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(54) Title: ANTIGENIC PROTEINS OF *BORRELIA BURGDORFERI*

(57) Abstract

The present invention relates to antigenic proteins specific to *Borrelia burgdorferi* which have a molecular weight of 28 kDa or 39 kDa as determined by SDS-PAGE and are reactive with Lyme borreliosis serum or fragments thereof and to the corresponding DNA. The proteins, especially the 39 kDa proteins (α and β) can be used to diagnose mammals previously or currently infected with the Lyme borreliosis causing agent.

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ANTIGENIC PROTEINS OF *BORRELIA BURGDORFERI*

FIELD OF THE INVENTION

The present invention relates to antigenic *Borrelia burgdorferi* proteins and their encoding DNA. In particular, the present invention relates to two 39 kilodalton (kDa) *Borrelia burgdorferi* proteins which react with Lyme borreliosis serum and a 28 kDa *Borrelia burgdorferi* protein which reacts with Lyme borreliosis serum.

BACKGROUND INFORMATION

Lyme borreliosis in humans is a multisystemic disorder caused by infection with the tick-borne spirochete, *Borrelia burgdorferi*, (Burgdorfer et al. 1982. Science 216:1317-1319; Johnson et al. 1984. Int. J. Syst. Bacteriol. 34:496-497; and Steere et al. 1983. N. Engl. J. Med. 308:733-740). Since the first epidemiological investigations of this disease in south-central Connecticut (Steere et al. 1977. Ann. Intern. Med. 86:685-698 and Steere et al. 1977. Arthritis. Rheum. 20:7-17), human cases of Lyme borreliosis have now been acquired in 43 states of the United States (Centers for Disease Control 1989, Lyme Disease - United States, 1987 and 1988. MMWR 38:668-672), five provinces of Canada, (Centers for Disease Control 1989, Lyme disease - Canada. MMWR 38:677-678), numerous countries throughout Europe and Asia (Ai et al. 1988. Ann. NY Acad. Sci. 539:302-313; Dekonenko et al. 1988. J. Infect. Dis. 158:748-753; and Schmid. 1985. Rev. Infect. Dis. 7:41-50), and possibly restricted foci in Australia (Stewart et al. 1982. Med. J. Australia 1:139) and Africa (Haberberger et al. 1989. Trans. R. Soc. Trop. Med. Hyg. 83:556 and Stanek et al. 1986. Zentralbl. Bakteriol. Mikrobio. Hyg. [A] 263:491-495). Between 1982-1988, reports of 13,825 cases of Lyme borreliosis were received by the Centers for Disease Control from all 50 states of the United States, (Centers for Disease Control 1989, Lyme Disease - United States, 1987 and 1988. MMWR 38:668-672), making this disease the most prevalent arthropod-borne infection in the country.

With the dramatic increase in awareness, prevalence, and geographical distribution of Lyme borreliosis, a tremendous new demand has been placed on clinical laboratories for serological confirmation of cases, (Magnarelli. 1989. J. Am. Med. Assoc. 262:3464-3465 and Schwartz et al. 1989. J. Am. Med. Assoc. 262:3431-3434) or to rule out this disease in differential diagnoses. However, many potential problems exist with the currently available serological tests for Lyme borreliosis, which may result in either false positive or false negative results (Magnarelli 1989. J. Am. Med. Assoc. 262:3464-3465). Some studies have focused on using flagellar protein of *B. burgdorferi* to increase the sensitivity of serological tests (Hansen et al. 1989. J. Clin. Microbiol 27:545-551 and Hansen et al. 1988. J. Clin. Microbiol 26:338-346) because earlier studies demonstrated that it appeared to be the 41 kilodalton (kDa) flagellar subunit (flagellin) of the spirochete that generated the earliest antibody response in infected humans (Barbour et al. 1983. J. Clin. Invest. 72:504-515; Coleman et al. 1987. J. Infect. Dis. 155:756-765; and Grodzicki et al. 1988. J. Infect. Dis. 157:790-797). One of two potential problems with using flagellar protein, however, is that flagella of other *Borrelia* species share epitopes common to the flagella of *B. burgdorferi* (Barbour et al. 1986. Infect. Immun. 52:549-544). Secondly, in most studies that have screened human sera by immunoblot analysis (Barbour. 1984. Yale J. Biol. Med. 57:581-586; Barbour et al. 1983. J. Clin. Invest. 72:504-515; Coleman et al. 1987. J. Infect. Dis. 155:756-765; Craft et al. 1986. J. Clin. Invest. 78:934-939; and Nadal et al. 1989. Pediatr. Res. 26:377-382), antibodies binding the protein with an apparent migration of 41 kDa have been assumed, but not proven, to be flagellin.

Thus, it is clear that a need exists for a method of detecting Lyme borreliosis disease in mammals. The present invention provides such a method.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a means for detecting mammals previously or presently infected with Lyme disease.

In one embodiment, the present invention relates to substantially pure forms of a *Borrelia burgdorferi* proteins which have molecular weights of about 39 kilodaltons and a protein which has a molecular weight of about 28 kilodaltons as determined by SDS-PAGE and which are reactive with Lyme borreliosis serum.

In another embodiment, the present invention relates to *Borrelia burgdorferi* proteins substantially free of proteins with which they are normally associated that have molecular weights of about 39 kilodaltons and a protein which has a molecular weight of about 28 kilodaltons as determined by SDS-PAGE and which are reactive with Lyme borreliosis serum.

In yet another embodiment, the present invention relates to a DNA fragment encoding all, or a unique portion, of the above described 39 kilodalton *Borrelia burgdorferi* proteins or the 28 kilodalton *Borrelia burgdorferi* protein.

In another embodiment, the present invention relates to a DNA fragment encoding all, or a unique portion, of one of the above described 39 kilodalton *Borrelia burgdorferi* proteins.

In a further embodiment, the present invention relates to a recombinant DNA molecule comprising a fragment of the above described DNA and a vector. The invention also relates to a host cell stably transformed with such a recombinant DNA molecule in a manner allowing expression of the *Borrelia burgdorferi* proteins encoded in the DNA fragment.

In another embodiment, the present invention relates to a method of producing recombinant *Borrelia burgdorferi* proteins of about 39 kilodaltons and a protein of about 28 kilodaltons and which are reactive with Lyme borreliosis serum which method comprises culturing host

cells expressing the proteins, in a manner allowing expression of the proteins, and isolating the proteins from the host cells.

In a further embodiment, the present invention relates to a purified form of an antibody specific for the above described 39 kilodalton *Borrelia burgdorferi* proteins or a unique fragment thereof or the above described 28 kilodalton *Borrelia burgdorferi* protein or a unique fragment thereof.

In another embodiment, the present invention relates to a vaccine for mammals against Lyme disease comprising all, or a unique portion, of the above described 39 kilodalton *Borrelia burgdorferi* proteins, each of the 39 kDa proteins or the above described 28 kilodalton protein *Borrelia burgdorferi* protein which are reactive with Lyme borreliosis serum, in an amount sufficient to induce immunization against Lyme disease, and a pharmaceutically acceptable carrier.

In a further embodiment, the present invention relates to a bioassay for the diagnosis of Lyme borreliosis disease comprising the steps of coating a surface with the 39 kDa proteins, each of the 39 kDa proteins or the 28 kDa protein of this invention (or antibodies specific therefor), contacting the surface with serum and detecting the presence or absence of a complex formed between the coated proteins (or coated antibodies) and antibodies specific therefor (or the target protein) in the serum.

In another embodiment, the present invention relates to a diagnostic kit comprising natural or recombinantly produced *Borrelia burgdorferi* 39 kDa proteins, each of the 39 kDa proteins or a 28 kDa protein and ancillary reagents suitable for use in detecting the presence of antibodies to the protein in a mammalian tissue sample.

In yet another embodiment, the present invention relates to a method of screening drugs for anti-Lyme borreliosis disease activity comprising contacting the

drug with cells contacted with *Borrelia burgdorferi* under conditions such that inhibition of anti-Lyme activity can be effected.

Various other objects and advantages of the present invention will become obvious from the drawings and the following description of the invention.

All publications mentioned herein are hereby incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows a genetic map of pSPR33. Spirochete DNA is denoted as a striped block and the arrow indicates the direction that the *Lac* promoter is transcribed. There were no restriction sites within the spirochete *Eco*RI fragment for *Acc*I, *Kpn*I, *Xba*I, *Xho*I or *Sma*I.

FIGURE 2 is an autoradiograph showing hybridization of ^{32}P-labeled insert DNA from pSPR33 with total DNA digested with *Eco*RI from 7 isolates of *B. burgdorferi* and 5 other *Borrelia* species. The right lane contained pSPR33 (pSPR33) digested with *Eco*RI. Linear molecular weight markers (Kb) are indicated on the right of the panel.

FIGURE 3 is an ethidium bromide stained gel of undigested total DNA from 7 isolates of *B. burgdorferi* (panel A) and an autoradiograph of the same gel after blotting to nitrocellulose and hybridization with the ^{32}P-labeled 6.3 Kb *Eco*RI fragment from pSPR33 (panel B). Note the strong hybridization signal associated with the chromosomal band.

FIGURE 4 shows immunoblot analysis of proteins expressed by pSPR33. Whole-cell lysates of *B. burgdorferi* strain Sh-2-82, *E. coli* carrying pSPR33 (*E. coli* + pSPR33) and *E. coli* carrying only vector (*E. coli* + vector) were immunoblotted with the human Lyme borreliosis serum used to screen the DNA library of *B. burgdorferi*.

FIGURE 5 demonstrates the specificity of P28 and P39 expression in *B. burgdorferi*. Whole-cell lysates of different *B. burgdorferi* strains (including low (P6) and

high (P246) *in vitro* passages of strain Sh-2-82) and isolates representing 5 additional *Borrelia* species were immunoblotted with anti-pSPR33 serum. Lysates of *E. coli* that express P28 and P39 (*E. coli* + pSPR33) and that do not (*E. coli* + vector) were also immunoblotted as positive and negative controls respectively. Not all of the 20 *B. burgdorferi* isolates tested are shown (see Table 1).

FIGURE 6 comprises the reactivity of anti-pSPR33 and monoclonal antibody H9724. Components in whole cell lysates of *E. coli* plus pSPR33, *E. coli* plus vector only, *B. burgdorferi* strain Sh-2-82 and *B.hermsii* strain FRG were separated by SDS-PAGE and were incubated with anti-pSPR33, anti-pSPR33 plus H9724, or H9724. P39 (arrow 1); 41 kDa flagellin from *B. burgdorferi* (arrow 2); 39 kDa flagellin from *B. hermsii* (arrow 3).

FIGURE 7 shows immunoblot analysis of 10 human Lyme borreliosis sera and their reactivity with P28 and P39. Whole-cell lysates of *B. burgdorferi* strain Sh-2-82 (lane 1), *E. coli* carrying pSPR33 (lane 2) and *E. coli* carrying only vector (lane 3) were immunoblotted with human Lyme borreliosis sera (NY). IFA Lyme borreliosis titers for each human serum are indicated below their designations. Autoradiographs exposed for 5 hr. (panel A) represented sera having weaker reactivity than those exposed for 1/2 hr. (panel B). Arrows denote P39 (arrow 1) and a 41 kDa antigen (arrow 2). Band B corresponds to the position of P28 and band A is an 58-65 kDa antigen that bound all sera that reacted with P39. Molecular mass markers (kDa) are indicated on the right of each panel.

FIGURE 8 shows immunoblot analysis of syphilitic sera. Whole-cell lysates of *B. burgdorferi* strain Sh-2-82, *E. coli* carrying pSPR33 and *E. coli* carrying only vector were immunoblotted with 5 syphilitic sera (1 to 5) or anti-pSPR33 (anti-pSPR33). Molecular mass markers are indicated on the right. Note absence of P39 in pSPR33 lanes reacted with syphilitic sera which contrasts with a strongly reactive 41 kDa antigen in three of the five *B. burgdorferi* lanes.

FIGURE 9 shows a restriction endonuclease map and expression data for the P39 locus of *Borrelia burgdorferi*. Subclones and deletion variants of plasmid pSPR33 (pSPR38, pSPR45, pSPR51, pSPR54, pSPR56, pSPR57, pSPR59, pSPR38, pSPR45, pSPR51, pSPR54, pSPR56, pSPR57, pSPR59, pSPR46, pSPR44 and pSPR42) are indicated as open bars.

FIGURES 10a and 10b show a map of the open reading frames of gene 1 and gene 2 encoding the P39 α and P39 β antigens, respectively, of *Borrelia burgdorferi*. Figure 10a shows the frames clear of termination sites (complete vertical lines). Figure 10b shows primer sites with overlapping sequences used to determine nucleotide sequences of both strands of DNA.

FIGURE 11 shows a genetic map of the P39 operon of *Borrelia burgdorferi* including the position of genes 1 and 2, the number of deduced amino acid, and the direction of transcription.

FIGURE 12 shows northern blot of *Borrelia burgdorferi* RNA probed with pSPR33 showing a 2.35 kb RNA transcript of the appropriate size for the single transcriptional unit for genes 1 and 2.

FIGURE 13 shows the deduced amino-terminal ends of P39 α (gene 1) and P39 β (gene 2), and the major outer surface proteins (Osp) A and B of *Borrelia burgdorferi*.

FIGURE 14 shows hydrophobicity plots of the deduced amino acid sequences of P39 α (dotted line) and P39 β (solid line) (Panel A) and OspA (dotted line) and OspB (solid line) (Panel B) of *Borrelia burgdorferi* (+ values show hydrophilic regions and - values show hydrophobic regions of the proteins).

DETAILED DESCRIPTION OF THE INVENTION

This invention relates, in part, to *Borrelia burgdorferi* antigenic proteins and their encoding DNA. A principle embodiment of this aspect of the present invention relates to three antigenic *Borrelia burgdorferi* proteins. Two proteins are characterized by a molecular weight of about 39 kDa (designated 39α and 39β) as determined by SDS-PAGE and reactivity with human Lyme

borreliosis serum. The third protein is characterized by a molecular weight of about 28 kDa as determined by SDS-PAGE and reactivity with human Lyme borreliosis serum. The present invention also relates to unique portions of the above proteins wherein a unique portion consists of at least 5 (or 6) amino acids.

The 39 kDa and 28 kDa proteins are substantially free of proteins with which they are normally associated. A substantially pure form of the proteins of the present invention can be obtained by one skilled in the art using standard methodologies for protein purification without undue experimentation. The present invention also relates to peptide fragments of the 39 kDa or 28 kDa protein. Alternatively, the proteins and peptides of the invention can be chemically synthesized using known methods.

The present invention also relates to a DNA fragment encoding all, or a unique portion, of the 39 kDa *B. burgdorferi* proteins or the 28 kDa *B. burgdorferi* protein of the present invention. A principle embodiment of this aspect of the invention relates to the 6.3 kilobase pair *Eco*RI fragment obtained from a DNA library of *B. burgdorferi* DNA which encodes the 39 kDa and 28 kDa antigenic proteins.

The present invention also relates to a DNA fragment encoating all, or a unique portion, of the 39 kDa α *B. burgdorferi* protein or the 39 kDa β *B. burgdorferi* protein.

The present invention further relates to a recombinant DNA molecule and to a host cell transformed therewith. Using standard methodology well known in the art, a recombinant DNA molecule comprising a vector and a DNA fragment encoding both the 39 kDa proteins of this invention, either of the 39 kDa proteins or the 28 kDa protein can be constructed using methods known in the art without undue experimentation. The DNA fragment can be isolated from *B. burgdorferi*, and it can take the form of a cDNA clone produced using methods well known to those skilled in the art or it can be produced by polymerase

chain reaction. Possible vectors for use in the present invention include, but are not limited to, λZAPII, pUC8 or preferably high frequency expression vectors such as pBluescript II SK, pNH8a. The host cell can be prokaryotic (such as bacterial), lower eukaryotic (such as fungal, including yeast) or higher eukaryotic (such as mammalian).

The present invention further relates to antibodies specific for the 39 kDa *B. burgdorferi* proteins or the 28 kDa protein of the present invention. One skilled in the art using standard methodology can raise monoclonal antibodies and polyclonal antibodies to the 39 kDa proteins or the 28 kDa protein, or a unique portion thereof. This is exemplified by the anti-pSPR33 rabbit antiserum (see Example 2 below).

The present invention also relates to a vaccine for use in mammals against Lyme borreliosis disease. In one embodiment of this aspect of this invention, as is customary for vaccines, the 39 kDa proteins, either of the 39 kDa proteins or the 28 kDa protein of the present invention can be delivered to a mammal in a pharmacologically acceptable vehicle. As one skilled in the art will understand, it is not necessary to use the entire protein. A unique portion of the protein (for example, a synthetic polypeptide corresponding to a portion of the 39 or 28 kDa proteins) can be used. Vaccines of the present invention can include effective amounts of immunological adjuvants known to enhance an immune response. The protein or polypeptide is present in the vaccine in an amount sufficient to induce an immune response against the antigenic protein and thus to protect against Lyme borreliosis infection. Protective antibodies are usually best elicited by a series of 2-3 doses given about 2 to 3 weeks apart. The series can be repeated when circulating antibodies concentration in the patient drops.

The present invention further relates to diagnostic assays for use in human and veterinary medicine. For diagnosis of Lyme borreliosis disease, the

presence of antibodies to the 39 kDa proteins or the presence of the 28 kDa proteins in mammalian serum is determined. Many types of tests, as one skilled in the art will recognized, can be used for detection. Such tests include, but are not limited to, IFA, RIA, RIST, ELISA, aggulination and hemagglutination. The diagnostic assays can be performed using standard protocols such as those described by Magnarelli et al. 1984. J. Clin. Microbiol. 20:181-184; Craft et al. 1984. J. Infect. Dis. 149:789-795; Enguall et al. 1971. Immunochemistry 8:871-874; and Russell et al. 1984. J. Infect. Dis. 149:465-470.

Specifically, a diagnostic assay of the present invention can be constructed by coating on a surface (ie. a solid support) for example, a microtitration plate or a membrane (eg. nitrocellulose membrane), all or a unique portion of the 39 kDa proteins (natural or synthetic), either of the 39 kDa proteins (natural or synthetic) or the 28 kDa protein (natural or synthetic) and contacting with the serum from a patient suspected of having Lyme borreliosis disease. The presence of a resulting complex formed between the surface and antibodies specific therefore in the serum can be detected by any of the known methods common in art, such as fluorescent antibody spectroscopy or colorimetry.

In another embodiment of the diagnostic assay of the present invention, all or a unique portion of the 39 kDa proteins, either of the 39 kDa proteins or the 28 kDa protein is bound to an inert particle of, for example, bentonite or polystyrene latex. The particles are mixed with serum from a patient in, for example, a well of a plastic agglutination tray. The presence or absence of antibodies in the patient's serum is determined by observing the settling pattern of the particles in the well.

In a further embodiment of the diagnostic assay of the present invention, the presence or absence of the 39 kDa proteins, or the 28 kDa protein in a serum sample is

detected. Antibodies specific for the 39 kDa proteins, either of the 39 kDa proteins or the 28 kDa protein or a unique portion thereof can be coated on to a solid surface such as a plastic and contacted with the serum sample. After washing, the presence or absence of the protein from the serum bound to the fixed antibodies is detected by addition of a labeled (e.g. fluorescently labeled) antibody specific for the 39 (or 28) kDa proteins.

One skilled in the art will appreciate that the invention includes the use of competition type assays in detecting in a sample the antigens and antibodies to which this invention relates.

The present invention further relates to screening for anti-Lyme borreliosis disease drugs. In one embodiment potential anti-Lyme borreliosis disease drugs are tested for their ability to inhibit expression of the 39 kDa proteins or the 28 kDa protein in cells contacted with the *B. burgdorferi*. The presence or absence of the 39 kDa proteins or 28 kDa protein in exposed cells treated with test drugs can be determined by any of the standard diagnostic assays mentioned above.

The present invention further relates to DNA fragments containing the nucleotide sequence as shown in Seq. Id No. 1-3, or mutants thereof, to recombinant molecules containing the DNA fragments and host cells transformed with the recombinant molecules. Using standard methodology well known in the art, a recombinant DNA molecule comprising a vector and the DNA fragments of this invention can be constructed using methods known in the art without undue experimentation. The DNA fragments can be isolated from *B. burgdorferi* or can be produced by a polymerase chain reaction. Possible vectors for use in the present invention include but are not limited to, pUC, pBluescript or pBR322. The host cell can be prokaryotic (such as bacterial), lower eukaryotic (such as fungal, including yeast) or higher eukaryotic (such as mammalian).

The present invention further relates to methods

of producing recombinant *Borrelia burgdorferi* 39 kDa and 28 kDa proteins comprising culturing the aforementioned host cells in a manner allowing expression of the proteins and. isolating the proteins from the host cells. Methodology utilize to produce recombinant *B. burgdorferi* proteins are well within the skill of an ordinary artisan.

EXAMPLES

The following organisms and materials were used throughout the Examples.

Bacterial strains. *B. burgdorferi* strains used (See Table 1 below) have been previously described or were kindly provided by Dr. John Anderson (Connecticut Agriculture Experiment Station, New Haven, Conn.), Dr. Alan MacDonald (Southampton Hospital, Long Island, N.Y.), and Ms. Glenna Teltow and Ms. Julie Rawlings (Medical Entomology Section, Bureau of Laboratories, Texas Department of Health, Austin, Tex.). The five strains representing *B. hermsii* (HS1), *B. coriaceae* (Co53), *B. parkeri*, *B. turicatae* and *B. anserina* have been described previously (Schwan et al. 1989. J. Clin. Microbiol. 27:1734-1738). Borrelia organisms were cultured at 32°C in BSK-II medium as previously described (Barbour. 1984. Yale J. Biol. Med. 57:581-586).

TABLE 1. Summary of *Borrelia burgdorferi* strains used in this study all of which expressed P28 and P39.

Strain designation	*In vitro*° passages (H)igh/(L)ow	Biological* source	Geographical* source (year isolated)	Obtained from (reference)
Sh-2-82	(P6) L	Id	New York (1982)	Schwan et al. (1)
Sh-2-82	(P246) H	Id	New York (1982)	Schwan et al. (1)
B31	H	Id	New York (1982)	Schwan et al. (1)
CA-2-87	L	Ip	California (1987)	Schwan et al. (1)

	CA-3-87 L	Ip	California (1987)	Schwan et al. (1)
	NY-1-86 L	H	New York (1986)	Schwan et al. (1)
	ECM-NY-86 L	H	New York (1986)	Schwan et al. (1)
	NY-6-86 L	H	New York (1982)	MacDonald
	NY-13-86 L	H	New York (1982)	MacDonald
	CT20004 L	Ir	France (1985)	Anderson
	CT22921 L	Rp	New York (1986)	Anderson
	CT26816 L	Rm	Rhode Island (1985)	Anderson
	CT19678 L	Rp	New York (1986)	Anderson
	CT21343 L	Rp	Wisconsin (1986)	Anderson
	CT21305 L	Rp	Connecticut (1986)	Anderson
	CT21721 L	Id	Wisconsin (1986)	Anderson
	CT27985 L	Id	Connecticut (1988)	Anderson
	TX1352 H	Aa	Texas (1989)	Rawlings
	PE92 H	D	Texas (1989)	Rawlings
	BR4-3028 H	H	Texas (1989)	Rawlings

[+]Tick = *Ixodes dammini* (Id); tick = *I. pacificus* (Ip); tick = *I. ricinus* (Ir); tick = *Amblyomma americanum* (Aa); human (H); rodent = *Peromyscus leucopus* (Rp); rodent = *Microtus* (Rm); dog = (D).
°Strains passed for ≤ 10 passages (L); strains passed for ≥ 20 passages (H).
*USA state or country.
1= Schwan et al. 1989. J. Clin. Microbiol. 27:1734,1738.

Human syphilitic sera were kindly provided by Dr. Wayne Hogefre and Ms. Jane Markley (Hillcrest Biologicals, Cypress, Calif.), amyotrophic lateral sclerosis (ALS) sera were provided by Dr. Jeffrey Smith (Mount Sinai Medical Center, ALS Clinic, New York, NY.) and Dr. Alan MacDonald (Southampton Hospital, Long Island, N.Y.), and relapsing fever sera were collected from patients from Oregon and Washington. Normal sera were obtained from staff and laboratory personnel at Rocky Mountain Laboratories. Human Lyme borreliosis sera were provided by Dr. Alan MacDonald and were collected from patients clinically diagnosed with Lyme borreliosis from Long Island, New York.

Escherichia coli carrying the plasmid pSPR33 (see below) were deposited on February 28, 1990 at the American Type Culture Collection 12301 Parklawn Drive, Rockville, Maryland 20852. The accession number of the organism is 68243. The deposits shall be viably maintaining, replacing it if it becomes non-viable, for the life of the patent, for a period of 30 years from the date of the deposit or for five years from the last date of request or sample of the deposit, whichever is longer and made available to the public upon issuance of a patent from this application, without restriction, and in accordance with the provisions of the law. The Commissioner of Patents and Trademarks, upon request shall have access to the deposit.

Example 1. Cloning and Genetic Analysis of *Borrelia* DNA

To identify *B. burgdorferi* proteins that induce an antibody response during the course of an infection, a DNA library of *B. burgdorferi* containing *Eco*RI fragments was constructed in *E. coli* with the λ expression vector λZAPII.

Total DNA was purified from 500 ml stationary phase borrelial cultures by a modification as previously described (Barbour. 1988. J. Clin. Microbiol. 26:475-478). Cells were recovered by centrifugation, washed in 20 ml of PBS plus 5 mM $MgCl_3$ and resuspended in 2.4 ml TES (50 mM Tris, pH 8.0; 50 mM EDTA, 15% (w/v) sucrose). Lysozyme was added to a final concentration of 1 mg/ml and then the cell suspension was left on ice for 10 min. Cells were lysed by adding 3 ml TES plus 1% (v/v) sodium deoxycholate and gently mixed for 10 min. at room temperature. Proteinase K (1 mg) was then added and the sample was incubated at 37°C for 1 hr. The DNA suspension was then extracted twice with 1 volume of phenol-chloroform (1:1 (v/v)) and once with chloroform-isoamyl alcohol (24:1 (v/v)). The DNA was ethanol precipitated, washed twice with 70% ethanol and resuspended to a final concentration of 1 mg/ml in TE (10 mM Tris, pH 7.6; 1 mM EDTA).

Total DNA (1 μg) from *B. burgdorferi* strain Sh-2-

82 was digested with *Eco*RI, ligated to the dephosphorylated arms of the expression vector λZAPII (Stratagene, La Jolla, Calif.) and packaged according to the manufacturer's directions.

The library was screened for *Borrelia* by immunoblot with a convalescent serum from a human Lyme borreliosis patient from Long Island, New York (1:100) following absorption of plaque proteins to nitrocellulose filters (Maniatis et al. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). After blocking for 1 hr. at 25°C in TSE-Tween (50 mM Tris, pH 7.4; 150 mM NaCl; 5 mM EDTA; 0.05% Tween 20), filters were incubated with serum diluted in TSE-Tween with gentle rocking at 25°C for 1 hr. They were then washed for 1 hr. with four changes of TSE-Tween and the bound antibody was detected by incubating the filters with ^{125}I-labeled protein A (500,000 cpm/ml) for 1 hr. with rocking. Each filter was then washed four times for 15 min. each with TSE-Tween, dried and autoradiographed with Kodak X-AR5 film.

Positive clones were detected at a frequency of 5%. One recombinant plaque that reacted with human serum was plaque purified and the phagemid carrying the *Borrelia* DNA was excised from the λ sequences with the aid of the helper phage R407 according to the suppliers directions (Stratagene). Excision of the cloned fragment from the purified phage produced the phagemid portion containing a 6.3 kilobase (Kb) *Eco*RI fragment, designated plasmid pSPR33 (Fig. 1). The fragment was isolated from an agarose gel, radiolabeled and shown to hybridize with a similar sized fragment in *Eco*RI digested total DNA from all six North American and one European *B. Burgdorferi* isolates (Fig. 2).

Recombinant plasmid pPSR33 was isolated from *E. coli* for mapping studies from 500 ml cultures and purified as previously described (Simpson et al. 1987. Infect. Immun. 55:2448-2455), except two consecutive dye-buoyant density gradients were preformed (Plasterk et al. 1985.

Nature 318:257-263) in a Beckman VTi80 rotary at 70,000 rpm for 4 hr at 18 C. The supercoiled circular plasmid portion was diluted with two volumes of water after the removal of the ethidium bromide and then ethanol precipitated. The plasmid DNA was then resuspended in a minimal volume of TE. Mini-plasmid preparations (Maniatis et al. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York) of positive clones were examined by agarose gel electrophoresis after their digestion with *Eco*RI to determine the insert size.

Southern blot analysis of undigested DNA from seven similar isolates, indicated that the 6.3 Kb fragment hybridized strongly with chromosomal DNA (Fig. 3). Undigested total DNA was electrophoresed in 0.4% agarose gels (12 v for 16 hrs). Southern blot procedures including the transfer of DNA from agarose gels to nitrocellose, high stringency hybridization (which permitted 10% basepair mismatch), and autoradiography were as previously described (Spanier et al. 1983. Virology 130:514-522) except that the prehybridization and hybridization buffers and temperatures were as described by Schwan et al. (Schwan et al. 1989. J. Clin. Microbiol. 27:1734-1738).

The DNA probe was recovered from agarose gels using Gene Clean (BIO 101, Inc., La Jolla, Calif.) and labeled with [α-^{32}P]dCTP (3,000 Ci/mmol) by nick translation according to the directions of the manufacturer (Nick Translation Kit, Bethesda Research Laboratories, Gaithersburg, Md.). The probe was boiled for 4 min. and quenched on ice immediately before adding to the hybridization buffer.

Restriction endonucleases were purchased from Boehringer Mannheim Biochemicals, Indianapolis, Ind., and digestions were performed as recommended by the manufacturer.

The smeared band in agarose-gels that contained heterogenous fragmented DNA and migrated slightly slower

than the 49 Kb linear plasmid from strain Sh-2-82 was assumed to be chromosomal DNA. Total DNA from 5 additional *Borrelia* species including *B. hermsii, B. parkeri, B. anserina, B. turicatae,* and *B. coriaceae* did not hybridize to the 6.3 Kb fragment (Fig. 2). These data indicate that the pSPR33 insert sequences are chromosomally located and are specific to *B. burgdorferi*.

Example 2. Immunoblot Analysis of Cloned *B. burgdorferi* Proteins

To identify the specific proteins encoded by pSPR33 that reacted with the human serum used to screen the library, whole-cell lysates of *E. coli* carrying pSPR33 were analyzed by SDS-PAGE and immunoblot.

Rabbit serum prepared against whole-cell lysates of *E. coli* carrying either pSPR33 (anti-pSPR33) or the vector pBluescript SK (anti-*E. coli*) were prepared as follows. Bacterial cells recovered from 16 hr cultures, were washed once and resuspended in phosphate buffered saline (PBS) to a final concentration of 10^8 cells/ml. The cells were killed by incubating for 30 min. at 56°C and disrupted by sonification on ice (2 min. at an output of 4; Branson Sonifier-Cell Disrupter 185). New Zealand white rabbits were immunized (without adjuvant) intramuscularly with 1.5 ml of the cell sonicate and boosted with the same immunogen at 21 and 42 days after the primary immunization. Sera were collected every 2 weeks thereafter for 4 months, pooled, and 5 ml aliquots absorbed with *E. coli* strain XL1-blue cells (Stratagene) collected from 500 ml cultures and incubated with rotation at 37°C for 4 hr. The bacteria were removed by centrifugation in a VTi80 rotor at 40,000 rpm for 30 min. This process was repeated twice, and absorbed sera were then filtered through a sterile 0.22 μm filter (Millipore Corp., Medford, Mass.) and stored at -20°C. Anti-pSPR33 and anti-*E. coli* sera was used at a dilution of 1:500 and 1:50 respectively. The monoclonal antibodies H5332 (Barbour et al. 1983. Infect. Immun. 41:795-804), H5TS (Barbour et al. 1984. Infect. Immun. 45:94-100), and

H9724 (Barbour et al. 1986. Infect. Immun. 52:549-554) were used at a dilution of 1:100.

IFA titers of Lyme borreliosis and relapsing fever sera were determined as previously described (Burgdorfer et al. 1982. Science 216:1317-1319). *B. burgdorferi* strain B31 and *B. hermsii* strain HS1 respectively were used as the antigens in the IFA tests.

Immunoblot analysis of whole-cell lysates were performed essentially as previously described (Schwan et al. 1989. Infect. Immun. 57:3445-3451) except cells were prepared as follows. Cells were recovered from liquid cultures by centrifugation (8,000 xg for 5 min), and resuspended in PBS to give an optical density of 0.2 at 600 nm. Cells from 2 ml of this suspension were recovered by centrifugation and resuspended in 100 µl of distilled water and 50 µl of sample buffer (0.2 M Tris, pH 6.8; 30% (v/v) glycerol; 3% (w/v) SDS; 0.002% (w/v) bromophenol blue). Samples were then boiled for 4 min. and 20 µl loaded onto a 12.5% SDS-PAGE gel. Gel electrophoresis, immunoblotting and detection of bound antibody, using ^{125}I protein A, have been described (Schwan et al. 1989. Infect. Immun. 57:3445-3451).

A 28 kDa (P28) and a 39 kDa (P39) antigen in the pSPR33 immunoblot profile, were the most immunoreactive antigens that were not detected in lysates of *E. coli* cells carrying only the vector (Fig. 4). Antisera raised to whole-cell lysates of *E. coli* carrying only the vector (anti-*E. coli* serum) did not react with P28 or P39 at a dilution of 1:50. These two proteins, therefore, are antigenically unrelated to native *E. coli* components and appear to be encoded by the cloned *Borrelia* sequences. P28 and P39 could not be resolved in SDS-PAGE gels stained with either Coomassie blue or silver nitrate because they co-migrate with other, more abundant *E. coli* proteins.

Similar sized proteins to P28 and P39 were detected by immunoblot (Fig. 4) in cell lysates of *B. burgdorferi* strain Sh-2-82, suggesting P28 and P39 are expressed by this strain. To determine if the 28 and 39

kDa *Borrelia* proteins seen in whole cell lysates were identical to the gene products P28 and P39 respectively, antiserum generated to cells carrying pSPR33 (anti-pSPR33 serum) was incubated with Western blotted whole-cell lysates of 1 European and 19 North American *B. burgdorferi* isolates, and compared to a lysate of *E. coli* producing P28 and P39 (Fig. 5). All of the 20 *Borrelia* isolates expressed a 39 kDa protein that co-migrated with P39. A 28 kDa protein was also detected, but considerably less antibody bound this protein than that which bound P39. P39 produced by pSPR33 also reacted with sera from five white-footed mice *(Peromyscus leucopus)* experimentally infected with *B. burgdorferi* strain Sh-2-82, but did not react with the preimmune sera from these animals, or with sera from mice infected only with *E. coli*. Other species of *Borrelia* did not produce detectable amounts of P28, P39 or any other antigenically related proteins under the conditions employed (Fig. 5). Extended exposure (> 24 hours) of autoradiographs revealed weak bands with molecular weights other than 28 kDa and 39 kDa in all *Borrelia* profiles, but these are attributed to non-specific binding. Data, including the fact that DNA from other species of *Borrelia* lacked sequences with close identity to those that encode P28 and P39 (Fig. 2), show that P28 and P39 are proteins specific to *B. burgdorferi*. Furthermore, anti-pSPR33 did not react with the *B. burgdorferi* antigens Osp A (31 kDa), Osp B (34 kDa) or the 41 kDa flagellin, suggesting that these proteins are antigenically unrelated to P28 and P39 (Fig. 5).

To confirm this, it was shown that the monoclonal antibodies H5332, H5TS and H9724 (Fig. 6), which bind specifically to Osp. A (Barbour et al. 1983. Infect. Immun. 41:795-804), Osp B (Barbour et al. 1984. Infect. Immun. 45:94-100) and the flagellin (Barbour et al. 1986. Infect. Immun. 52:549-554) respectively, did not bind to P28 or P39 produced by either pSPR33 or strain Sh-2-82. The specificity of monoclonal antibody H9724 for *Borrelia* flagellin is evident in Figure 6, as this monoclonal only

bound a 41 kDa band in the *B. burgdorferi* profile and a 39 kDa band, which corresponds to its flagellin (Barbour et al. 1986. Infect. Immun. 52:549-554), in the *B. hermsii* profile. Furthermore, using electron microscopy and colloidal gold staining, monoclonal antibody H9724 bound to endoflagellin from *B. burgdorferi* whereas anti-pSPR33 did not.

Example 3. Immunoreactivity of Lyme Borreliosis Sera with Cloned Borrelia Proteins

To test the possibility that P28 and P39 are immunodominant proteins, ninety-four human sera collected from patients clinically diagnosed as having Lyme borreliosis were tested for reactivity with cloned P28 and P39 at a dilution of 1:100. Whole-cell lysates were electrophoresed in SDS-PAGE gels and Western blotted as previously described in the above Examples. The nitrocellulose was cut into equal strips (5 per gel) such that each strip contained lanes for *E. coli* carrying pSPR33, *E. coli* carrying only the vector and *B. burgdorferi* strain Sh-2-82. Each strip was incubated with a different human serum except for one strip from each gel which was incubated with anti-pSPR33 serum. This latter strip served as marker for the positions of P28 and P39. All of 33 sera with IFA titers ≥ 1:256 (100%), 13 of 17 sera (76%) with IFA titers = 1:128, and 14 of 44 sera (32%) with titers ≤ 1:64 reacted with P39 (see Table 2 below).

Table 2. Summary of human Lyme borreliosis sera tested for reactivity with P39

IFA Titer Percent Sera Positive	No. Sera Tested	No. Sera Reacting with P39
≥ 1:2048 100	5	5

1:1024 100	8	8
1:512 100	9	9
1:256 100	11	11
1:128 76	17	13
1:64 40	10	4
1:32 55	9	5
≤ 1:16 20	25	5
Total	94	60

Examples of immunoblots for human sera reacting with P39 (arrow 1) are shown in Figure 7. A strongly reacting 58-65 kDa band was observed in the *B. burgdorferi* profile (Fig. 7, band A) for all sera that reacted with P39, but since anti-pSPR33 serum does not react to a band in this region of the gel (Fig. 5), P39 and the 58-65 kDa protein(s) are presumably unrelated. Although P28 appeared to react strongly to some sera (Fig. 7B, band B), for other, less reactive sera, it was not clear if the sera reacted to P28 or to some other protein. This was because these sera also reacted with co-migrating *E. coli* proteins that were detected with a longer autoradiographic exposure (Fig. 7A, band B). Therefore, although it is not clear to what extent P28 actually reacts with human Lyme borreliosis sera, it appears that antibody to P39 was detected in 100% of all sera that had IFA titers ≥ 1:256. Notably, many sera reactive to P39 did not appear to react with the 41 kDa flagellin (Fig. 7A & 7B). In view of this, antibody to P39 could be mistaken as antibody to the flagellin when testing human sera by immunoblot using whole-cell lysates of *B. burgdorferi*. Because P39 was

shown to be specific to *B. burgdorferi* by immunoblot, it is not surprising that control sera, which included sera from 5 ALS patients, 5 syphilitic patients, 5 relapsing fever patients and 10 normal individuals who showed no symptoms of clinical disease, did not react to the cloned P39 protein at a dilution of 1:50 (see Table 3 below). Immunoblot findings for the syphilitic sera are shown in Figure 8. These data suggest that P39 has antigenic specificity for sera collected from patients with Lyme borreliosis. This is despite the fact that both the syphilitic and relapsing fever sera tested had significantly high IFA Lyme borreliosis titers (see Table 3 below), and therefore most likely contained cross-reacting antibodies directed at other *B. burgdorferi* antigens.

Table 3. Summary of IFA titers for control sera that did not react with P39.

Serum description plasma	Lyme IFA	Relapsing fever IFA	Rapid reagin test (1)
Syphilitic			
1	1:128	1:256	1:128
2	1:256	1:1024	1:128
3	1:1024	1:2048	1:128
4	1:512	1:1024	1:64
5	1:128	1:1024	1:32
Relapsing fever			
1	1:1024	1:1024	---
2	1:32	1:512	---
3	1:128	1:512	---
4	1:64	1:512	---
5	1:64	1:1024	---
ALS			
1	1:16	1:64	---
2, 3, 4	<1:16	<1:16	---
5	1:16	1:16	---

Normal			
1, 2, 3, 4	<1:16	1:16	---
5, 6, 7, 8	<1:16	<1:16	---
9, 10	<1:16	1:32	---

1=Portnoy, 1963. Amer. J. Clin. Pathol. 40:473-479

The immunodominance of P39 and this antigens' potential to be a virulence factor of *B. burgdorferi* on account of its immune characteristics and association with infectivity, lead to further characterization of the genetic basis for P39 expression.

Example 4. PSPR 33 Subclone and deletion analysis. Eleven subclones were constructed to determine the approximate position of the P39 locus in the 6.3 kb EcoRI insert in the parent construct pSPR33. The endonucleases EcoRI, ClaI, HindIII, BamHI and PstI were used according to the manufacturer (Boehringer Mannheim Biochemicals) to produce various restriction fragments, which were then ligated to the linearized pBluescript cloning vector (Stratagene) cut with the appropriate enzyme or combination of enzymes. A 4.4 kb EcoRI - ClaI fragment was ligated into the vector and transformed into DH5 alpha Escherichia coli competent cells (Bethesda Research Laboratories) and designated pSPR38 (Fig. 9). A 2.3 kb EcoRI - HindIII fragment produced the subclone pSPR45; a 5.0 kb BamHI fragment produced the subclone pSPR46; a 3.3 kb PstI fragment produced the subclone pSPR44; a 1.4 kb PstI fragment produced the subclone pSPR42. Additional subclones were produced as deletion products by deleting sequences from the HindIII end of the EcoRI - HindIII DNA fragment in the subclone pSPR45. Once digested with HindIII, Dnase was applied for increasing lengths of time to shorten the fragment. The new end was treated with DNA polymerase and nucleotides were added to blunt the end for ligation into linearized, blunt-ended vectors (pBluescript). By successive treatments, the subclones pSPR51, pSPR54, pSPR57, and pSPR59 were

constructed (Fig. 9).

To determine whether the clones were expressing P39, expression assays of the P39 deletion and subclone variants (Fig. 9) were performed with polyclonal anti-P39 serum (anti-pSPR33, previously described), monoclonals A6 and D1 and Western blotted whole-cell lysates. Two monoclonal antibodies to P39 antigen were produced using standard techniques for one of ordinary skill in the art. Escherichia coli cells containing the recombinant pSPR33 were inoculated intraperitoneally into BALB/c laboratory mice. After one month, the mice were boosted with an identical inoculum. One week after the boost, serum samples from the mice were tested by Western blot analysis for anti-P39 antibodies and mice seropositive were again boosted with recombinant E. coli. After three days, spleen were removed. Spleen cells were separated and fused with hybridoma cells SP-20 in HY culture media, 37° C, 8% CO_2. Successful fusions were then cloned by limiting dilution in 96-well microtiter plates. Tissue culture supernatants of positive cell cultures were then tested by Western blot analysis for anti-P39 antibody. Two clones positive for such analysis, designated A6 and D1, were used in subsequent analysis of P39 antigen and the expression of various subclones of pSPR33 as previously described.

To examine various antisera and monoclonal antibodies by Western blot analysis for anti-P39 antibodies, the *E.* coli recombinant with pSPR33 was first lysed by heat in 2-mercaptoethanol and then electrophoresed in a 12.5% SDS-polyacrylamide gel for 6 hr. The gel was then electroblotted with the Towbin system for 3 hr. to transfer the *E. coli* recombinant proteins onto a nitrocellulose membrane. After transfer, the membrane was blocked with TSE-Tween to reduce the nonspecific binding of immunoglobulins. Next the membrane was immersed in the appropriate test serum or monoclonal antibody and incubated at room temperature with rocking for 1 hr. The membrane was then rinsed with water and

incubated next in a solution of ^{125}I-protein A to label antibodies bound to the antigens on the membrane. After incubation and washing off the excess label, the membrane was dried and placed on Kodak XAR-5 film for autoradiographic detection of the anti-P39 antibodies. Similar assays were conducted for the other subclones.

Plasmids pSPR38 and pSPR46 expressed the same amount of P39 as the primary clone pSPR33. This, along with the fact that plasmid pSPR51 expressed P39 whereas pSPR54 did not, we conclude that the gene for P39 was between the RamHI and HindIII sites (Fig. 9, black bar). The amount of P39 associated with cell lysates of clones pSPR51 and pSPR45 is less than the other clones that were P39 positive. This suggested that sequences to the right of the gene locus were important for full expression. P39 was produced by a clone (pSPR46) that contained the insert in the opposite orientation to that of other P39 producing clones (e.g. pSPR38). Therefore, expression or P39 was not dependent on the Lac promoter (Fig. 9, back arrows). The pst1 fragment that was subcloned from pSPR33 and designated pSPR44 (Fig. 9), did not express detectable amounts of P39. Thus, the P39 gene was assumed to be transcribed from left to right. We presume that the additional sequences correspond to the second of two genes that express similar but distinct antigens and that they collectively augment the amount of antibody that binds the 39 kDa band in the immunoblot assay. Because the plasmid pSPR44 did not express any antigens reactive with polyclonal anti-P39 serum, the expression of the second gene located to the right of the black box (Fig. 9) may depend on the transcription of the first gene.

Example 5. <u>DNA sequencing of the gene encoding P39</u>. The DNA sequence (Fig. 10) was determined for the <u>BamHI-HindIII</u> fragment (Fig. 9, black box) by the strategy summarized in Fig. 11b. Essentially, sequence was obtained using primers designed from DNA sequence determined using the universal M13 primer and the subclones pSPR46, pSPR44, and pSPR45, and the Mung Bead

nuclease deletion variants pSPR51, pSPR54, pSPR56, and pSPR57, of plasmid pSPR45. DNA sequence to the right of the HindIII restriction site was determined using primers designed from existing sequence information.

DNA sequence was obtained first by using primers designed for use with the M13 universal primer and available sequence of the cloning vector. The protocol for performing the sequencing reactions was exactly that provided by United States Biochemical (Sequenase - Version 2.0: Step-By-Step Protocols for DNA Sequencing With Sequenase ® Version 2.0 - 5th Edition). Sequencing reactions were run in small plastic centrifuge tubes. Each reaction volume was 10Nl and included primer, buffer and DNA to anneal primer to template. Labeling was done by adding Sequenase, ^{35}S-dATP, and additional buffer. Termination of the A, T, G, and C reactions was done by adding a stop solution. Samples were then heated to 70° - 80°C for two minutes and then 2-3 Nl of each mix was added to each lane of the gel. All sequencing gels were 6% acrylamide - 7M urea - 1 x TBE and were run for 2hr or 4hr. After running, the gels were fixed in 5% acetic acid - 15% methanol to remove urea. Gels were then dried at 80°C under vacuum then placed on Kodak XAR-5 film. Exposed films were then analyzed for autoradiographic bands to determine the sequence. Terminal sequences of each reaction were used to generate new oligonucleotide primers for use in the next sequencing reactions. Therefore, the entire sequences of each strand of DNA were determined through successive extensions using primers determined by previous reactions. By way of example, synthetic primers of 20 nucleotides from a region of SEQ ID no. 1 can be constructed and utilized to sequence about 300 bases. Other primers can then be constructed from the deduced sequence. Such techniques are standard and would be known to one of ordinary skill in the art.

Analysis of the completed DNA sequence (SEQ. ID No. 1) revealed two open reading frames (Fig. 10a). Gene 1 was in frame 1 and gene 2 was in frame 3. No other

significant open reading frames were detected. The DNA sequence has been numbered from the adenine residue of the ATG start codon for the protein encoded by gene 1 because it is assumed that this is the first gene transcribed. This open reading frame (nucleotides 1 to 1020) was confirmed by sequencing the first 15 amino acids of P39 expressed by clone pSPR51. This clone has had gene 2 deleted, and therefore its gene product was not detected during protein sequencing. Gene 1 corresponds to a protein of 339 amino acids with a calculated molecular weight of 36.926 kDa. Because this gene encodes a protein that reacted with all of 10 serum specimens collected from: human Lyme patient but not to 10 normal controls specimens (data not shown), it assumed that this protein is equivalent to P39. Because of the existence of a second gene product with a similar molecular weight that may also bind human serum, it was determined that the P39 antigen as previously described is not one protein but two proteins (39α and 29 β). This is suggested by the expression data shown in Fig. 9, where the P39 signal appears to be enhanced if both genes are present. The open reading frame (nucleotides 1107 to 2132) of gene 2 has been designated p39β. This genes' open reading frame begins 116 nucleotides down stream of p39α and encodes a protein of 341 amino acids (37.506 kDa). A promoter 5' to the start codon in p39α appeared to be present with classic -10 and -35 regions whereas the p39β lacked recognizable promoter sequences. Both genes, however, had putative ribosomal binding sites immediately 5' to the start codons and each was terminated with a TAA codon at positions 1018 and 2130 respectively. The putative promoter and ribosomal binding sites resemble those associated with other genes from *B. burgdorferi* including the opsA-operon and the flagellin gene (Wallich, R., S.E. Moter, M.M. Simon, K. Ebnet, A. Heiberger, and M.D. Kramer, 1990. The *Borrelia burgdorferi* flagellum-associated 41 kilodalton antigen (flagellin); molecular cloning, expression and amplification of the gene. Infect

Immun. 58:1711-1719). Unlike the genes encoding the flagellin, OspA and OspB, no stem loop structures were detected at the 3' 'end of either p39α and p39β, suggesting termination may be outside what has been sequenced. Nevertheless, in accordance with the transcription termination regions in may bacteria, including *Borrelia*, this region is AT rich, suggesting that termination is in the vicinity of nucleotide 2170.

Comparing the DNA sequence of p39α and p38β by the Needleman and Munsch global alignment program (Needleman and Munsch, J. Mol. Biol.; 148:443-53 (1970)), indicates that these genes have 62% DNA sequence similarity. No significant sequence similarity was detected between the P39 genes and either the OspA-OspB operon or the flagellin gene. Codon preference and G + C content analysis of the p39 operon indicated that there were no significant differences between it and the other *Borrelia* genes.

Example 6. Determination of p39α and p39β transcript size. Northern blot analysis (Fig. 12) of total RNA from *B. burgdorferi* strains B31 and Sh-2-82 were probed with a PstI-HindIII fragment internal to the p39α and p39β loci (Fig. 11). This probe detected a single 2.35 kb message, and tends to confirm that the P39α and β mRNA is polycistronic and that p39α and p39β constitute an operon (p39). This conclusion is supported by the DNA sequence data described above which shows that p39β does not appear to have a recognizable promoter. Furthermore, this explains why clones that carry an intact p39β but lack the promoter for p39α (eg. pSPR44), do not express antigens reactive with polyclonal anti-P39 serum (anti-pSPR33) (Fig. 9). As a control for specificity, total RNA from E. coli was shown not to hybridize to the PstI-HindIII *Borrelia* fragment (Fig. 12). The amino acid composition of P39α and P39β are similar (SEQ. ID. No. 2 and 3, Table 4), although distinct from the amino acid composition of OspA and OspB. P39 and P39β contained comparatively much larger amounts of isoleucine, proline, arginine, phenylalanine, tyrosine, and methionine.

Furthermore, lysine and threonine, which are present in large amounts in OspA and OspB, constitute a much smaller proportion of P39α and P39β. Between P39α and P39β, the major difference was the 3 cysteine residues in the later protein and 4 histidine residues in the former protein (Table 4).

Table 4. Amino acid composition of proteins encoded by the P39 operon

	P39α(%)	P39β(%)
Alanine	25 (7.4)	22 (6.5)
Cysteine	1 (0.3)	3 (0.9)
Aspartic acid	20 (5.9)	21 (6.2)
Glutamic acid	26 (7.7)	21 (6.2)
Phenylalanine	16 (4.7)	16 (4.7)
Glycine	33 (9.7)	32 (9.4)
Histidine	4 (1.2)	1 (0.3)
Isoleucine	37 (10.9)	43 (7.6)
Lysine	30 (8.8)	26 (7.6)
Leucine	32 (9.4)	26 (7.6)
Methionine	5 (1.5)	6 (1.8)
Asparagine	17 (5.0)	21 (6.2)
Proline	8 (2.4)	7 (2.1)
Glutamine	4 (1.2)	7 (2.1)
Arginine	7 (2.1)	9 (2.6)
Serine	29 (8.6)	30 (8.8)
Threonine	12 (3.5)	5 (1.5)
Valine	18 (5.3)	25 (7.3)
Tryptophan	1 (0.3)	2 (0.6)
Tyrosine	14 (4.1)	18 (5.3)
	339	341

* * * * * *

P39β, line OspA and OspB, has a classic signal peptide including the putative cleavage site defined by the tetrapeptide Leu-X-X-Cys (Fig. 13), where X usually represents any neutral amino acid. For P39β, the leu residue is at position 12 and the cysteine at position 15

(SEQ. ID. No. 1). Although P39α also has a hydrophobic N-terminus (Fig. 14) and a cysteine at a similar position (position 18), this protein does not have the tetrapeptide, suggesting that its putative signal sequence is processed in a different manner to that of the corresponding region in P39β. Because P39α and P39β have a cysteine at close to the same position as the cysteine in OspA and OspB, and it has been predicted that the latter two proteins are acylated at the site, P39α and P39β may also be lipoproteins due to acylation of their N-terminal cysteine residue.

Comparing the amino acid sequence of P39α and P39β revealed 52% sequence identity. This is similar to the reported 53% similarity between OspA and OspB. Surprisingly and in contrast to that found for OspA and OspB, the p39 operon proteins have very similar hydropathy plots (Fig. 14). This, along with the high degree of sequence similarity, indicates that the two proteins share a considerable number of the same epitopes having immunogenic properties. Antiserum raised to OspA will react to OspB, indicating proteins like P39α and P39β with significant identity at the amino acid level will share cross-reactive epitopes.

The genetic element encoding the immunodominate antigen P39 was identified and sequenced. This element was shown to be two genes that constituting an operon encoding two similar sized proteins, P39α and P39β, that have considerably amino acid sequence similarity. This is the first report of an operon encoding putative membrane proteins that has a chromosomal origin in *B. burgdorferi*. It is assumed that both the α and β forms contribute to the signal when antibody from infected animals binds the P39 band in Western blots (Simpson, W.J., W. Burgdorfer, M.E. Schrumpf, R.H. Karstens, and T.G. Schwan, 1991. Antibody to a 39 kDa *Borrelia burgdorferi* antigen (P39) as a marker for infection in experimentally and naturally inoculated animals. J Clin Microbio 29:236-243. Simpson, W.J., M.E. Schrumpf, and T.G. Schwan, 1990. Reactivity of

human Lyme borreliosis sera with a 39-kilodalton antigen specific to *Borrelia burgdorferi*. *J Clin Microbiol 28:1329-1337*). This raises the question of whether all Lyme serum reacts equally well to both α and β forms or whether some serum reacts to one and not the other.

The function of P39α and P39β is not known, but several characteristics, deduced from the predicted amino acid sequence suggests several possibilities. These proteins exhibit alternating hydrophobic and hydrophilic regions, characteristic of an amphophilic or transmembrane protein. In accordance with a membrane location, immune electron microscopy analyses of *B. burgdorferi* with monoclonal antibody A6 indicates that the P39 antigen is in or associated with the membranes (unpublished data).

P39β resembles OspA and OspB in that it has typical signal sequence and cleavage site at the first cysteine residue. Like OspA and OspB, P39β is probably membrane associated and may be acrylated at the N-terminal cysteine. P39α, however, is different with regard to its signal sequence which may not be cleaved because it lacks the type 1 recognition site. If so, P39α may be secreted and therefore the antigen that stimulates the immune response during an infection. This notion would help to explain the earlier observation that anti-P39 antibodies appear to more readily associated with the infected state, because a secreted form could accumulate more rapidly during the early stages of an infection than that associated with cells. (Simpson, W.J., W. Burgdorfer, M.E. Schrumpf, R.H. Karstens, and T.G. Schwan, 1991. Antibody to a 39 kDa *Borrelia burgdorferi* antigen (P39) as a marker for infection in experimentally and naturally inoculated animals. J Clin Microbiol 29:236-243.)

Example 6: <u>Amplification of gene 1 (P39α) and gene 2 (P39β)</u>

To determine the immunoreactiveness both P39α and P39β, gene 1 and gene 2 will be cloned separately and the expression products examined for their reactivity with Lyme immune sera. Standard methodologies for cloning and

expressing each gene can be employed; however, it is preferred to amplify each gene separately using the polymerase chain reactive (PCR) and the primer sequences identified in SEQ. ID Nos. 4-7.

The synthetic oligonucleotide DNA primers described were constructed with an Applied Biosystems Inc. DNA Synthesizer Model 380-B, following the instructions provided by the manufacturer. In this procedure, short chains of nucleotides of a specific order are produced in a concentrated ammonium hydroxide solution. This material is then centrifuged under vacuum to remove the ammonium hydroxide. The dry DNA pellet is then resuspended in TE buffer and the DNA concentration is determined by spectrophotometric absorbance at 260 mm. Concentrations of the DNA primers are then standardized for PCR according to the protocol provided by Perkin-Elmer-Cetus.

To amplify *B. burgdorferi* DNA by PCR using the primers described, the protocol involves mixing the *B. burgdorferi* DNA with either primers 1 and 2 for gene 1 (sequences 4 and 5), primers 1 and 2 for gene 2 (sequences 6 and 7), and sequences 4 and 7 to amplify both genes 1 and 2 together. Also added to the PCR mix is the DNA *Taq* polymerase, buffer, and the mixture of the four nucleotides (dNTPs). This reaction mixture is then subjected to repetitive cycles of three different temperatures to cause denaturing the DNA, annealing of the primers to the template DNA, and extension (polymerization) to produce a new strand of DNA. After 30 cycles using the thermal cycler, the PCR amplification products are examined by running 10 μl of each sample in an electrophoresis agarose gel.

In order that the amplified products can be inserted into known vectors by standard techniques known to a skilled artisan, at the 5' end of each primer, nucleotides will be added that encode for the recognition site for the restriction endonuclease EcoRI (G/AATTC).

The amplified DNA products will be comprised of each gene with the addition of an EcoRI site at each end,

which will allow us to insert this sequence into any one of many available cloning and expression vectors which have only one EcoRI site available, such as pUC, pBluescript, pBR322, etc. The vectors are inserted into host cells to obtain expression of the DNA products. Such techniques are well known to one of ordinary skill in the art.

Next, recombinants having the appropriate sized inserted DNA (1017 bases for gene 1; 1023 bp for gene 2) will be examined by Southern blot analysis to identify the cloned fragments. DNA from recombinants with the presumptive gene 1 or gene 2 will be separated in agarose gels, transferred to nitrocellulose membranes, and probed with the purified EcoRI fragment from pSPR33. Such procedures are standard techniques well known to anyone skilled in the art. After confirming that the amplified cloned fragments are homologous with the pSPR33 insert, the various clones are tested for expression of P39 antigens using standard Western immunoblotting techniques. Rabbit anti-pSPR33 antiserum, anti-P39 monoclonal antibodies (as previously described), and convalescent serum from human Lyme patients will each be reacted with whole-cell lysates of the various clones to identify and obtain expression products of each gene. The synthetic peptides can be mapped to identify specific immunoreactive epitopes, used in bioassays to detect Lyme borreliosis disease or used in vaccines for mammals against Lyme borreliosis disease.

While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be appreciated by one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention and appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(ii) TITLE OF INVENTION: ANTIGENIC PROTEINS OF BORRELIA BURGDORFERI

(iii) NUMBER OF SEQUENCES: 7

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE:

(B) STREET:

(C) CITY:

(D) STATE:

(E) COUNTRY:

(F) ZIP:

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette - 3.5 inches, 1.44 Mb storage

(B) COMPUTER: IBM PC Compatible

(C) OPERATING SYSTEM: MS-DOS

(D) SOFTWARE: WordPerfect 5.0

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME:

(B) REGISTRATION NUMBER:

(C) REFERENCE/DOCKET NUMBER:

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE:

(B) TELEFAX:

(C) TELEX:

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2,307

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: double stranded

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Genomic DNA

(A) DESCRIPTION:

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Borrelia burgdorferi

(B) STRAIN: Sh-2-82

(C) INDIVIDUAL ISOLATE: Sh-2-82, P-6

(D) DEVELOPMENTAL STAGE:

(E) HAPLOTYPE:

(F) TISSUE TYPE:

(G) CELL TYPE:

(H) CELL LINE:

(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:

(A) LIBRARY:

(B) CLONE: pSPR33

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT:

(B) MAP POSITION:

(C) UNITS:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(C) IDENTIFICATION METHOD: By experiment

(D) OTHER INFORMATION: Expression of P-39 antigens

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NUMBER:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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TCCTGATAGT GAATATGCAT TTGATTTATT TAAATCAAAG TTATAAACTA CTAAATATAG   60
                        -35                     -10

CTTTGTTTGT AAAGGGGAAA TAGTTT ATG AAT AAA ATA TTG TTG TTG ATT TTG   113
                             Met Asn Lys Ile Leu Leu Leu Ile Leu
                                                   1

CTT GAG AGT ATT GTT TTT TTA TCT TGT AGT GGT AAA GGT AGT CTT GGG   161
Leu Glu Ser Ile Val Phe Leu Ser Cys Ser Gly Lys Gly Ser Leu Gly
10                                      20

AGC GAA ATT CCT AAG GTA TCT TTA ATA ATT GAT GGA ACT TTT GAT GAT   209
Ser Glu Ile Pro Lys Val Ser Leu Ile Ile Asp Gly Thr Phe Asp Asp
            30                                          40

AAA TCT TTT AAT GAG AGT GCT TTA AAT GGC GTA AAA AAA GTT AAA GAA   257
Lys Ser Phe Asn Glu Ser Ala Leu Asn Gly Val Lys Lys Val Lys Glu
                                50

GAA TTT AAA ATT GAG CTT GTT TTA AAA GAA TCC TCA TCA AAT TCT TAT   305
Glu Phe Lys Ile Glu Leu Val Leu Lys Glu Ser Ser Ser Asn Ser Tyr
        60                                      70

TTA TCT GAT CTT GAA GGG CTT AAG GAT GCG GGC TCA GAT TTA ATT TGG   353
Leu Ser Asp Leu Glu Gly Leu Lys Asp Ala Gly Ser Asp Leu Ile Trp
                        80

CTT ATT GGG TTT TAT AGA TTT AGC GAT GTG GCC AAG GTT GCG GCT CTT   401
Leu Ile Gly Phe Tyr Agr Phe Ser Asp Val Ala Lys Val Ala Ala Leu
90                                              100

CAA AAT CCC GAT ATG AAA TAT GCA ATT ATT GAT CCT ATT TAT TCT AAC   449
Gln Asn Pro Asp Met Lys Tyr Ala Ile Ile Asp Pro Ile Tyr Ser Asn
                    110                                     120

GAT CCT ATT CCT GCA AAT TTG GTG GGC ATG ACC TTT AGA GCT GAA GAG   497
Asp Pro Ile Pro Ala Asn Leu Val Gly Met Thr Phe Arg Ala Gln Glu
                                130

GGT GCA TTT TTA ACG GGT TAT ATT GCT GCA AAA CTT TCT AAA ACA GGT   545
Gly Ala Phe Leu Thr Gly Tyr Ile Ala Ala Lys Leu Ser Lys Thr Gly
            140                                     150
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AAA ATT GGA TTT TTA GGG GGA ATA GAA GGC GAG ATA GTA GAT GCT TTT   593
Lys Ile Gly Phe Leu Gly Gly Ile Glu Gly Glu Ile Val Asp Ala Phe
                            160

AGG TAT GGG TAT GAA GCT GGT GCT AAG TAT GCT AAT AAA GAT ATA AAG   641
Arg Tyr Gly Tyr Glu Ala Gly Ala Lys Try Ala Asn Lys Asp Ile Lys
    170                                 180

ATA TCT ACT CAG TAT ATT GGT AGT TTT GCT GAC CTT GAA GCT GGT AGA   689
Ile Ser Thr Gln Tyr Ile Gly Ser Phe Ala Asp Leu Glu Ala Gly Arg
                    190                                     200

AGC GTT GCA ACT AGA ATG TAT TCT GAT GAG ATA GAC ATT ATT CAT CAT   737
Ser Val Ala Thr Arg Met Try Ser Asp Glu Ile Asp Ile Ile His His
                                210

GCT GCA GGC CTT GGA GGA ATT GGG GCT ATT GAG GTT GCA AAA GAA CTT   785
Ala Ala Gly Leu Gly Gly Ile Gly Ala Ile Glu Val Ala Lys Glu Leu
            220                                 230

GGT TCT GGG CAT TAC ATT ATT GGA GTT GAT GAA GAT CAA GCA TAT CTT   833
Gly Ser Gly His Tyr Ile Ile Gly Val Asp Glu Asp Gln Ala Tyr Leu
                        240

GCT CCT GAC AAT GTA ATA ACA TCT ACA ACT AAA GAT GTT GGT AGA GCT   881
Ala Pro Asp Asn Val Ile Thr Ser Thr Thr Lys Asp Val Gly Arg Ala
    250                                 260

TTA AAT ATT TTT ACA TCT AAC CAT TTA AAA ACT AAT ACT TTC GAA GGT   929
Leu Asn Ile Phe Thr Ser Asn His Leu Lys Thr Asn Thr Phe Glu Gly
                    270                                     280

GGC AAA TTA ATA AAT TAT GGC CTT AAA GAA GGA GTT GTG GGG TTT GTA   977
Gly Lys Leu Ile Asn Tyr Gly Leu Lys Glu Gly Val Val Gly Phe Val
                                290

AGA AAT CCT AAA ATG ATT TCC TTT GAA CTT GAA AAA GAA ATT GAC AAT  1025
Arg Asn Pro Lys Met Ile Ser Phe Glu Leu Glu Lys Glu Ile Asp Asn
            300                                 310

CTT TCT AGC AAA ATA ATC AAC AAA GAA ATT ATT GTT CCA TCT AAT AAA  1073
Leu Ser Ser Lys Ile Ile Asn Lys Glu Ile Ile Val Pro Ser Asn Lys
                        320

GAA AGT TAT GAG AAG TTT CTT AAA GAA TTT ATT TAA ATAAAGAATC AATTTATATA
1129  Gly Ser Tyr Glu Lys Phe Leu Lys Glu Phe Ile ***
          330

TTTTATTTTT AAGTATAAAA AACACATTGG TTTTGTTTGA ATAATTGAAA TGGAGAAGTG 1189

CTTTAT ATG AGA ATT GTA ATT TTT ATA TTC GGT ATT TTT TTG ACT TCT    1237
       Met Arg Ile Val Ile Phe Ile Phe Gly Ile Leu Leu Thr Ser
       1                                   10
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TGC TTT AGT AGA AAT GGA ATA GAA TCT AGT TCA AAA AAA ATT AAG ATA   1285
Cys Phe Ser Arg Asn Gly Ile Gly Ser Ser Ser Lys Lys Ile Lys Ile
                    20                                      30

TCC ATG TTG GTA GAT GGT GTT CTT GAC GAC AAA TCT TTT AAT TCT AGT   1333
Ser Met Leu Val Asp Gly Val Leu Asp Asp Lys Ser Phe Asn Ser Ser
                                40

GCT AAT GAG GCT TTA TTA CGC TTG AAA AAA GAT TTT CCA GAA AAT ATT   1381
Ala Asn Glu Ala Leu Leu Arg Leu Lys Lys Asp Phe Pro Glu Asn Ile
            50                                          60

GAA GAA GTT TTT TCT TGT GCT ATT TCT GGA GTT TAT TCT AGT TAT GTT   1429
Glu Glu Val Phe Ser Cys Ala Ile Ser Gly Val Tyr Ser Ser Tyr Val
                            70

TCA GAT CTT GAT AAT TTA AAA AGG AAT GGC TCA GAC TTG ATT TGG CTT   1477
Ser Asp Leu Asp Asn Leu Lys Arg Asn Gly Ser Asp Leu Ile Trp Leu
    80                                      90

GTA GGG TAC ATG CTT ACG GAT GCA TCT TTA TTG GTT TCA TCG GAG AAT   1525
Val Gly Tyr Met Leu Thr Asp Ala Ser Leu Leu Val Ser Ser Glu Asn
                100                                         110

CCA AAA ATT AGC TAT GGA ATA ATA GAT CCC ATT TAT GGT GAT GAT GTT   1573
Pro Lys Ile Ser Tyr Gly Ile Ile Asp Pro Ile Tyr Gly Asp Asp Val
                                120

CAG ATT CCT GAA AAC TTG ATT GCT GTT GTT TTC AGA GTA GAG CAA GGT   1621
Gln Ile Pro Glu Asn Leu Ile Ala Val Val Phe Arg Val Glu Gln Gly
            130                                         140

GCT TTT TTG GCT GGC TAT ATT GAC GCC AAA AAA AGC TTT TCT GGC AAA   1669
Ala Phe Leu Ala Gly Thr Ile Ala Ala Lys Lys Ser Phe Ser Gly Lys
                            150

ATA GGT TTT ATA GGG GGA ATG AAG GGT AAT ATA GTA GAT GCA TTT CGC   1717
Ile Gly Phe Ile Gly Gly Met Lys Gly Asn Ile Val Asp Ala Phe Arg
                                    170

ACT GGT TAT GAA TCT GGA GCA AAG TAT GCT AAT AAA GAT ATA GAG ATT   1765
Thr Gly Tyr Glu Ser Gly Ala Lys Tyr Ala Asn Lys Asp Ile Glu Ile
                180                                         190

ATA AGT GAA TAT TCC AAT TCT TTT TCC GAT GTT GAT ATT GGT AGA ACC   1813
Ile Ser Glu Tyr Ser Asn Ser Phe Ser Asp Val Asp Ile Gly Arg Thr
                                200

ATA GCT AGT AAA ATG TAT TCT AAA GGG ATA GAT GTA ATT CAT TTT GCA   1861
Ile Ala Ser Lys Met Tyr Ser Lys Gly Ile Asp Val Ile His Phe Ala
            210                                         220

GCT GGT TTA GCA GGA ATT GGT GTT ATT GAG GCA GCA AAA AAC CTT GGC   1909
Ala Gly Leu Ala Gly Ile Gly Val Ile Glu Ala Ala Lys Asn Leu Gly
                            230
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GAT GGT TAC TAT GTT ATT GGA GCC GAT CAG GAT CAG TCA TAT CTT GCT   1957
Asp Gly Tyr Tyr Val Ile Gly Ala Asp Gln Asp Gln Ser Tyr Leu Ala
    240                             250

CCT AAA AAT TTT ATT ACT TCT GTT ATA AAA AAC ATT GGG GAC GCA TTG   2005
Pro Lys Asn Phe Ile Thr Ser Val Ile Lys Asn Ile Gly Asp Ala Leu
                    260                                     270

TAT TTG ATT ACT GGC GAA TAT ATT AAA AAT AAT AAT GTT TGG GAA GGT   2053
Tyr Leu Ile Thr Gly Glu Tyr Ile Lys Asn Asn Asn Val Trp Glu Gly
                                280

GGA AAA GTT GTT CAA ATG GGA TTA AGA GAT GGT GTT ATT GGG CTG CCT   2101
Gly Lys Val Val Gln Met Gly Leu Arg Asp Gly Val Ile Gly Leu Pro
            290                                 300

AAT GCG AAT GAA TTT GAA TAC ATA AAA GTT CTT GAG AGA AAA ATA GTC   2149
Asn Ala Asn Glu Phe Glu Tyr Ile Lys Val Leu Glu Arg Lys Ile Val
                        310

AAT AAA GAG ATC ATT GTT CCT TGC AAT CAG GAG GAA TAT GAA ATT TTT   2197
Asn Lys Glu Ile Ile Val Pro Cys Asn Gln Glu Glu Tyr Glu Asn Phe
    320                                 330

ATA AAA CAA ATA TTA AAG TTA TAA ACTTTTGAAA TAGAAAGATT TTAATTTTCC  2251
Ile Lys Gln Ile Leu Lys Leu ***
                    340

AGTTTTTAAT TTTTTAATTA TGTTATATTT ATTGTGTTAT AATAAATAGA AGTACA     2307
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1109

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: double stranded

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Genomic DNA

(A) DESCRIPTION:

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Borrelia burgdorferi

(B) STRAIN: Sh-2-82

(C) INDIVIDUAL ISOLATE: Sh-2-82, P-6

(D) DEVELOPMENTAL STAGE:

(E) HAPLOTYPE:

(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE: pSPR33

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD: By experiment
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
TCCTGATAGT GAATATGCAT TTGATTTATT TAAATCAAAG TTATAAACTA CTAAATATAG  60
                     -35                          -10

CTTTGTTTGT AAAGGGGAAA TAGTTT ATG AAT AAA ATA TTG TTG TTG ATT TTG  113
                              Met Asn Lys Ile Leu Leu Leu Ile Leu
                              1

CTT GAG AGT ATT GTT TTT TTA TCT TGT AGT GGT AAA GGT AGT CTT GGG   161
Leu Glu Ser Ile Val Phe Leu Ser Cys Ser Gly Lys Gly Ser Leu Gly
10                                  20
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```
AGC GAA ATT CCT AAG GTA TCT TTA ATA ATT GAT GGA ACT TTT GAT GAT   209
Ser Glu Ile Pro Lys Val Ser Leu Ile Ile Asp Gly Thr Phe Asp Asp
                30                                          40

AAA TCT TTT AAT GAG AGT GCT TTA AAT GGC GTA AAA AAA GTT AAA GAA   257
Lys Ser Phe Asn Glu Ser Ala Leu Asn Gly Val Lys Lys Val Lys Glu
                                    50

GAA TTT AAA ATT GAG CTT GTT TTA AAA GAA TCC TCA TCA AAT TCT TAT   305
Glu Phe Lys Ile Glu Leu Val Leu Lys Glu Ser Ser Ser Asn Ser Tyr
        60                                              70

TTA TCT GAT CTT GAA GGG CTT AAG GAT GCG GGC TCA GAT TTA ATT TGG   353
Leu Ser Asp Leu Glu Gly Leu Lys Asp Ala Gly Ser Asp Leu Ile Trp
                            80

CTT ATT GGG TTT TAT AGA TTT AGC GAT GTG GCC AAG GTT GCG GCT CTT   401
Leu Ile Gly Phe Tyr Agr Phe Ser Asp Val Ala Lys Val Ala Ala Leu
90                                              100

CAA AAT CCC GAT ATG AAA TAT GCA ATT ATT GAT CCT ATT TAT TCT AAC   449
Gln Asn Pro Asp Met Lys Tyr Ala Ile Ile Asp Pro Ile Tyr Ser Asn
                    110                                     120

GAT CCT ATT CCT GCA AAT TTG GTG GGC ATG ACC TTT AGA GCT GAA GAG   497
Asp Pro Ile Pro Ala Asn Leu Val Gly Met Thr Phe Arg Ala Gln Glu
                                130

GGT GCA TTT TTA ACG GGT TAT ATT GCT GCA AAA CTT TCT AAA ACA GGT   545
Gly Ala Phe Leu Thr Gly Tyr Ile Ala Ala Lys Leu Ser Lys Thr Gly
            140                                         150

AAA ATT GGA TTT TTA GGG GGA ATA GAA GGC GAG ATA GTA GAT GCT TTT   593
Lys Ile Gly Phe Leu Gly Gly Ile Glu Gly Glu Ile Val Asp Ala Phe
                        160

AGG TAT GGG TAT GAA GCT GGT GCT AAG TAT GCT AAT AAA GAT ATA AAG   641
Arg Tyr Gly Tyr Glu Ala Gly Ala Lys Try Ala Asn Lys Asp Ile Lys
    170                                             180

ATA TCT ACT CAG TAT ATT GGT AGT TTT GCT GAC CTT GAA GCT GGT AGA   689
Ile Ser Thr Gln Tyr Ile Gly Ser Phe Ala Asp Leu Glu Ala Gly Arg
                    190                                     200


AGC GTT GCA ACT AGA ATG TAT TCT GAT GAG ATA GAC ATT ATT CAT CAT   737
Ser Val Ala Thr Arg Met Try Ser Asp Glu Ile Asp Ile Ile His His
                                210

GCT GCA GGC CTT GGA GGA ATT GGG GCT ATT GAG GTT GCA AAA GAA CTT   785
Ala Ala Gly Leu Gly Gly Ile Gly Ala Ile Glu Val Ala Lys Glu Leu
            220                                         230

GGT TCT GGG CAT TAC ATT ATT GGA GTT GAT GAA GAT CAA GCA TAT CTT   833
Gly Ser Gly His Tyr Ile Ile Gly Val Asp Glu Asp Gln Ala Tyr Leu
                        240
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GCT CCT GAC AAT GTA ATA ACA TCT ACA ACT AAA GAT GTT GGT AGA GCT   881
Ala Pro Asp Asn Val Ile Thr Ser Thr Thr Lys Asp Val Gly Arg Ala
    250                                     260

TTA AAT ATT TTT ACA TCT AAC CAT TTA AAA ACT AAT ACT TTC GAA GGT   929
Leu Asn Ile Phe Thr Ser Asn His Leu Lys Thr Asn Thr Phe Glu Gly
                    270                                     280

GGC AAA TTA ATA AAT TAT GGC CTT AAA GAA GGA GTT GTG GGG TTT GTA   977
Gly Lys Leu Ile Asn Tyr Gly Leu Lys Glu Gly Val Val Gly Phe Val
                                    290

AGA AAT CCT AAA ATG ATT TCC TTT GAA CTT GAA AAA GAA ATT GAC AAT  1025
Arg Asn Pro Lys Met Ile Ser Phe Glu Leu Glu Lys Glu Ile Asp Asn
            300                                     310

CTT TCT AGC AAA ATA ATC AAC AAA GAA ATT ATT GTT CCA TCT AAT AAA  1073
Leu Ser Ser Lys Ile Ile Asn Lys Glu Ile Ile Val Pro Ser Asn Lys
                            320

GAA AGT TAT GAG AAG TTT CTT AAA GAA TTT ATT TAA                  1109
Gly Ser Tyr Glu Lys Phe Leu Lys Glu Phe Ile ***
```

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1198

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: double stranded

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Genomic DNA

(A) DESCRIPTION:

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Borrelia burgdorferi

(B) STRAIN: Sh-2-82

(C) INDIVIDUAL ISOLATE: Sh-2-82, P-6

(D) DEVELOPMENTAL STAGE:

(E) HAPLOTYPE:

(F) TISSUE TYPE:

(G) CELL TYPE:

(H) CELL LINE:

(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE: pSPR33
(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

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                                                    ATAAAGAATC AATTTATATA  20

TTTTATTTTT AAGTATAAAA AACACATTGG TTTTGTTTGA ATAATTGAAA TGGAGAAGTG  80

CTTTAT ATG AGA ATT GTA ATT TTT ATA TTC GGT ATT TTT TTG ACT TCT      128
       Met Arg Ile Val Ile Phe Ile Phe Gly Ile Leu Leu Thr Ser
       1                                   10
TGC TTT AGT AGA AAT GGA ATA GAA TCT AGT TCA AAA AAA ATT AAG ATA     176
Cys Phe Ser Arg Asn Gly Ile Gly Ser Ser Ser Lys Lys Ile Lys Ile
                    20                                  30

TCC ATG TTG GTA GAT GGT GTT CTT GAC GAC AAA TCT TTT AAT TCT AGT    224
Ser Met Leu Val Asp Gly Val Leu Asp Asp Lys Ser Phe Asn Ser Ser
                                    40

GCT AAT GAG GCT TTA TTA CGC TTG AAA AAA GAT TTT CCA GAA AAT ATT    272
Ala Asn Glu Ala Leu Leu Arg Leu Lys Lys Asp Phe Pro Glu Asn Ile
            50                                          60
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```
GAA GAA GTT TTT TCT TGT GCT ATT TCT GGA GTT TAT TCT AGT TAT GTT   320
Glu Glu Val Phe Ser Cys Ala Ile Ser Gly Val Tyr Ser Ser Tyr Val
                         70

TCA GAT CTT GAT AAT TTA AAA AGG AAT GGC TCA GAC TTG ATT TGG CTT   368
Ser Asp Leu Asp Asn Leu Lys Arg Asn Gly Ser Asp Leu Ile Trp Leu
 80                                  90

GTA GGG TAC ATG CTT ACG GAT GCA TCT TTA TTG GTT TCA TCG GAG AAT   416
Val Gly Tyr Met Leu Thr Asp Ala Ser Leu Leu Val Ser Ser Glu Asn
            100                                             110

CCA AAA ATT AGC TAT GGA ATA ATA GAT CCC ATT TAT GGT GAT GAT GTT   464
Pro Lys Ile Ser Tyr Gly Ile Ile Asp Pro Ile Tyr Gly Asp Asp Val
                    120

CAG ATT CCT GAA AAC TTG ATT GCT GTT GTT TTC AGA GTA GAG CAA GGT   512
Gln Ile Pro Glu Asn Leu Ile Ala Val Val Phe Arg Val Glu Gln Gly
        130                                 140

GCT TTT TTG GCT GGC TAT ATT GAC GCC AAA AAA AGC TTT TCT GGC AAA   560
Ala Phe Leu Ala Gly Thr Ile Ala Ala Lys Lys Ser Phe Ser Gly Lys
                150

ATA GGT TTT ATA GGG GGA ATG AAG GGT AAT ATA GTA GAT GCA TTT CGC   608
Ile Gly Phe Ile Gly Gly Met Lys Gly Asn Ile Val Asp Ala Phe Arg
 160                                 170

TAT GGT TAT GAA TCT GGA GCA AAG TAT GCT AAT AAA GAT ATA GAG ATT   656
Thr Gly Tyr Glu Ser Gly Ala Lys Tyr Ala Asn Lys Asp Ile Glu Ile
            180                                             190

ATA AGT GAA TAT TCC AAT TCT TTT TCC GAT GTT GAT ATT GGT AGA ACC   704
Ile Ser Glu Tyr Ser Asn Ser Phe Ser Asp Val Asp Ile Gly Arg Thr
                    200

ATA GCT AGT AAA ATG TAT TCT AAA GGG ATA GAT GTA ATT CAT TTT GCA   752
Ile Ala Ser Lys Met Tyr Ser Lys Gly Ile Asp Val Ile His Phe Ala
        210                                 220

GCT GGT TTA GCA GGA ATT GGT GTT ATT GAG GCA GCA AAA AAC CTT GGC   800
Ala Gly Leu Ala Gly Ile Gly Val Ile Glu Ala Ala Lys Asn Leu Gly
                230

GAT GGT TAC TAT GTT ATT GGA GCC GAT CAG GAT CAG TCA TAT CTT GCT   848
Asp Gly Tyr Tyr Val Ile Gly Ala Asp Gln Asp Gln Ser Tyr Leu Ala
 240                                 250

CCT AAA AAT TTT ATT ACT TCT GTT ATA AAA AAC ATT GGG GAC GCA TTG   896
Pro Lys Asn Phe Ile Thr Ser Val Ile Lys Asn Ile Gly Asp Ala Leu
            260                                             270

TAT TTG ATT ACT GGC GAA TAT ATT AAA AAT AAT AAT GTT TGG GAA GGT   944
Tyr Leu Ile Thr Gly Glu Tyr Ile Lys Asn Asn Asn Val Trp Glu Gly
                    280
```

```
GGA AAA GTT GTT CAA ATG GGA TTA AGA GAT GGT GTT ATT GGG CTG CCT    992
Gly Lys Val Val Gln Met Gly Leu Arg Asp Gly Val Ile Gly Leu Pro
            290                                 300

AAT GCG AAT GAA TTT GAA TAC ATA AAA GTT CTT GAG AGA AAA ATA GTC   1040
Asn Ala Asn Glu Phe Glu Tyr Ile Lys Val Leu Glu Arg Lys Ile Val
                        310

AAT AAA GAG ATC ATT GTT CCT TGC AAT CAG GAG GAA TAT GAA ATT TTT   1088
Asn Lys Glu Ile Ile Val Pro Cys Asn Gln Glu Glu Tyr Glu Asn Phe
    320                                 330

ATA AAA CAA ATA TTA AAG TTA TAA ACTTTTGAAA TAGAAAGATT TTAATTTTCC  1142
Ile Lys Gln Ile Leu Lys Leu ***
                    340

AGTTTTTAAT TTTTTAATTA TGTTATATTT ATTGTGTTAT AATAAATAGA AGTACA     1198
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(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single-Stranded

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Synthetic DNA

(A) DESCRIPTION: A primer from the flanking sequence of 5' to gene 1 of P-39 in B. burgdorferi.

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM: B. burgdorferi

(B) STRAIN: Sh-2-82

(C) INDIVIDUAL ISOLATE: Sh-2-82, P-6

(D) DEVELOPMENTAL STAGE:

(E) HAPLOTYPE:

(F) TISSUE TYPE:

(G) CELL TYPE:

(H) CELL LINE:

(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:

(A) LIBRARY:

(B) CLONE: pSPR33

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT:

(B) MAP POSITION:

(C) UNITS:

(ix) FEATURE: A primer from the flanking DNA of 5' to gene 1 of P-39 in B. burgdorferi.

(A) NAME/KEY:

(B) LOCATION:

(C) IDENTIFICATION METHOD:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NUMBER:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

ATG AAT AAA ATA TTG TTG 18

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single-Stranded

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Synthetic DNA

(A) DESCRIPTION: A primer from within the insertion sequence of genes 1 and 2 of P-39 in B. burgdorferi.

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Borrelia burgdorferi
(B) STRAIN: Sh-2-82
(C) INDIVIDUAL ISOLATE: Sh-2-82, P-6
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE: pSPR33

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE: A primer from within the insertion sequence of genes 1 and 2 of P-39 in B. burgdorferi.
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:
AAT AAA TTC TTT AAG AAA 18

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single-Stranded

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Synthetic DNA

(A) DESCRIPTION: A primer from within the insertion sequence of genes 1 and 2 of P-39 in B. burgdorferi.

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Borrelia burgdorferi

(B) STRAIN: Sh-2-82

(C) INDIVIDUAL ISOLATE: Sh-2-82, P-6

(D) DEVELOPMENTAL STAGE:

(E) HAPLOTYPE:

(F) TISSUE TYPE:

(G) CELL TYPE:

(H) CELL LINE:

(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:

(A) LIBRARY:

(B) CLONE: pSPR33

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT:

(B) MAP POSITION:

(C) UNITS:

(ix) FEATURE: A primer from within the insertion sequence of genes 1 and 2 of P-39 in B. burgdorferi.

(A) NAME/KEY:

(B) LOCATION:

(C) IDENTIFICATION METHOD:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

ATG AGA ATT GTA ATT TTT 18

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single-Stranded
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Synthetic DNA
(A) DESCRIPTION: A primer from within the insertion sequence of genes 1 and 2 of P-39 in B. burgdorferi.

(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Borrelia burgdorferi
(B) STRAIN: Sh-2-82
(C) INDIVIDUAL ISOLATE: Sh-2-82, P-6
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE: pSPR33

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT:

(B) MAP POSITION:

(C) UNITS:

(ix) FEATURE: A primer from within the insertion sequence of 5' to gene 2 of P39 in B. Burgdorferi.

(A) NAME/KEY:

(B) LOCATION:

(C) IDENTIFICATION METHOD:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NUMBER:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

TAA ATT TAA TAT TTG TTT 18

WHAT IS CLAIMED IS:

1. A substantially pure form of a *Borrelia burgdorferi* protein which has a molecular weight of about 39 kilodalton as determined by SDS-PAGE and which is reactive with mammalian Lyme borreliosis serum.

2. The protein according to claim 1 wherein said protein is P39α or P39β.

3. The protein according to claim 1 wherein the mammal is a human.

4. A substantially pure form a *Borrelia burgdorferi* protein which has a molecular weight of about 28 kilodaltons as determined by SDS-PAGE and which is reactive with mammalian Lyme borreliosis serum.

5. The protein according to claim 4 wherein the mammal is a human.

6. A *Borrelia burgdorferi* protein substantially free of proteins with which it is normally associated that has a molecular weight of about 39 kilodaltons as determined by SDS-PAGE and which is reactive with mammalian Lyme borreliosis serum.

7. The protein according to claim 6 wherein said protein is P39α or P39β.

8. The protein according to claim 6 wherein the mammal is a human.

9. A *Borrelia burgdorferi* protein substantially free of proteins with which it is normally associated that has a molecular weight of about 28 kilodaltons as determined by SDS-PAGE and which is reactive with mammalian Lyme borreliosis serum.

10. The protein according to claim 9 wherein the mammal is a human.

11. A DNA fragment encoding all, or an unique portion, of a *Borrelia burgdorferi* protein, which protein has a molecular weight of about 39 kilodaltons as determined by SDS-PAGE and is reactive with mammalian Lyme borreliosis serum.

12. The DNA fragment according to claim 11 wherein said protein is P39α or P39β.

13. A DNA fragment encoding all, or a unique portion, of *Borrelia burgdorferi* proteins 39α and P39β and which are reactive with mammalian Lyme borreliosis serum.

14. A DNA fragment encoding all, or an unique portion, of a *Borrelia burgdorferi* protein, which protein has a molecular weight of about 28 kilodaltons as determined by SDS-PAGE and is reactive with mammalian Lyme borreliosis serum.

15. A recombinant DNA molecule comprising:
1) said DNA fragment according to claim 11; and
2) a vector.

16. A recombinant DNA molecule comprising:
1) said DNA fragment according to claim 12; and
2) a vector.

17. A recombinant DNA molecule comprising:
1) said DNA fragment according to claim 13, and
2) a vector.

18. A recombinant DNA molecule comprising:
1) said DNA fragment according to claim 14, and
2) a vector.

19. The recombinant DNA molecule according to claim 15 wherein said vector is pBluescript SK.

20. The recombinant DNA molecule according to claim 18 wherein said vector is pBluescript SK.

21. The recombinant DNA molecule according to claim 15 which is pSPR33.

22. A host cell stably transformed with the recombinant DNA molecule according to claim 15 in a manner allowing expression of said protein encoded in said DNA fragment.

23. A host cell stably transformed with the recombinant DNA molecule according to claim 16 in a manner allowing expression of said protein encoded in said DNA fragment.

24. A host cell stably transformed with the recombinant DNA molecule according to claim 17 in a manner allowing expression of said proteins encoded in said DNA fragment.

25. A host cell stably transformed with the recombinant DNA molecule according to claim 18 in a manner allowing expression of said proteins encoded in said DNA fragment.

26. The host cell according to claim 22 wherein said host cell is *Escherichia coli*.

27. The host cell according to claim 18 wherein said host cell is *Escherichia coli*.

28. A method of producing a recombinant *Borrelia burgdorferi* 39 kD protein comprising culturing host cells according to claim 22, in a manner allowing expression of said 39 kD protein, and isolating said 39 kD protein from said host cells.

29. A method of producing a recombinant *Borrelia burgdorferi* 39 kDa protein comprising culturing host cells according to claim 23, in a manner allowing expression of said 39 kD protein, and isolating said 39 kD protein.

30. A method of producing recombinant *Borrelia burgdorferi* 39α and 39β kD proteins comprising culturing host cells according to claim 24, in a manner allowing expression of said 39α and 39β proteins, and isolating said 39α and 39β proteins.

31. A method of producing a recombinant *Borrelia burgdorferi* 28 kD protein comprising culturing host cells according to claim 25, in a manner allowing expression of said 28 kD protein, and isolating said 28 kD protein from said host cells.

32. A complex comprising said protein according to claim 1 bound to a solid support.

33. A purified form of an antibody specific for said protein according to claim 1, or a unique fragment thereof.

34. A purified form of an antibody specific for said protein according to claim 2, or a unique fragment thereof.

35. A purified form of an antibody specific for said protein according to claim 4, or a unique fragment thereof.

36. The antibody according to claim 33 which is monoclonal.

37. The antibody according to claim 35 which is monoclonal.

38. The antibody according to claim 33 which is polyclonal.

39. The antibody according to claim 35 which is polyclonal.

40. A complex comprising said antibody according to claim 33 bound to a solid support.

41. A complex comprising said antibody according to claim 35 bound to a solid support.

42. A vaccine for mammals against Lyme borreliosis disease comprising all, or a unique portion, of a 39 kD *Borrelia burgdorferi* protein, in an amount sufficient to induce immunization against said disease, and a pharmaceutically acceptable carrier.

43. The vaccine according to claim 42 wherein said protein is P39α or P39β.

44. A vaccine for mammals against Lyme borreliosis disease comprising all, or a unique portion, of 39 kD α and β proteins, in an amount sufficient to induce immunization against said disease, and a pharmaceutically acceptable carrier.

45. A vaccine for mammals against Lyme borreliosis disease comprising all, or a unique portion, of a 28 kD *Borrelia burgdorferi* protein, in an amount sufficient to induce immunization against said disease, and a pharmaceutically acceptable carrier.

46. The vaccine according to claim 42 which further comprises an adjuvant.

47. The vaccine according to claim 43 which further comprises an adjuvant.

48. The vaccine according to claim 45 which further comprises an adjuvant.

49. A bioassay for the diagnosis of Lyme borreliosis disease comprising the steps of:

i) coating a surface with all, or a unique portion, of the protein according to claim 1;

ii) contacting said coated surface with serum from a mammal suspected of having Lyme disease; and

iii) detecting the presence or absence of a complex

formed between said protein and antibodies specific therefor present in said serum.

50. The bioassay according to claim 29 wherein said protein is P39α or P39β.

51. A bioassay for the diagnosis of Lyme borreliosis disease comprising the steps of:

i) coating a surface with all, or a unique portion, of the protein according to claim 4;

ii) contacting said coated surface with serum from a mammal suspected of having Lyme disease; and

iii) detecting the presence or absence of a complex formed between said protein and antibodies specific therefor present in said serum.

52. The method according to claim 49 wherein said surface is a gel, a slide, membrane or a microtitration plate.

53. A bioassay for the diagnosis of Lyme borreliosis disease comprising the steps of:

i) coating a surface with antibodies according to claim 33;

ii) contacting said coated surface with serum from a mammal suspected of having Lyme disease; and

iii) detecting the presence or absence of a complex formed between said antibodies and proteins present the said serum.

54. A bioassay for the diagnosis of Lyme borreliosis disease comprising the steps of:

i) coating a surface with antibodies according to claim 34;

ii) contacting said coated surface with serum from a mammal suspected of having Lyme disease; and

iii) detecting the presence or absence of a complex formed between said antibodies and proteins present the said serum.

55. A bioassay for the diagnosis of Lyme borreliosis disease comprising the steps of:

i) coating a surface with antibodies according to claim 35;

ii) contacting said coated surface with serum from a mammal suspected of having Lyme disease; and

iii) detecting the presence or absence of a complex formed between said antibodies and proteins present the said serum.

56. A diagnostic kit comprising a natural or recombinantly produced *Borrelia burgdorferi* 39 kDa proteins and ancillary reagents suitable for use in detecting the presence of antibodies to said proteins in a mammalian tissue sample.

57. The diagnostic kit according to claim 56 wherein said protein is P39α or P39β.

58. The diagnostic kit according to claim 56 wherein said tissue sample to be tested is a blood sample.

59. A method of screening drugs for anti-Lyme borreliosis disease activity comprising contacting said drug with cells contacted with *Borrelia burgdorferi* under conditions such that inhibition of said anti-Lyme activity can be effected.

60. A DNA fragment comprising the nucleotide sequence shown in SEQ ID No. 1 or a mutant thereof.

61. A DNA fragment comprising the nucleotide type sequence shown in SEQ ID No. 2 or a mutant thereof.

62. A DNA fragment comprising the nucleotide sequence shown in SEQ ID No. 3 or a mutant thereof.

63. A recombinant molecule comprising:

1) said DNA fragment according to claim 60; and

2) a vector.

64. A recombinant molecule comprising:

1) said DNA fragment according to claim 61; and

2) a vector.

65. A recombinant molecule comprising:

1) said DNA fragment according to claim 62; and

2) a vector.

66. A host stably transformed with the recombinant DNA molecule according to claim 63 in a matter allowing expression of said proteins encoded in said DNA fragment.

67. A host stably transformed with the recombinant DNA

molecule according to claim 64 in a matter allowing expression of said protein coated in said DNA fragment.

68. A host stably transformed with the recombinant DNA molecule according to claim 65 in a matter allowing expression of said protein coated in said DNA fragment.

69. A method of producing a recombinant *Borrelia burgdorferi* 39 kilodalton proteins comprising culturing host cells according to claim 66, in a manner allowing expression of said proteins, and isolating said proteins from said host cells.

70. A method of producing a recombinant *Borrelia burgdorferi* 39 kilodaltons α protein comprising culturing host cells according to claim 67, in a manner allowing expression of said 39 kilodalton α protein, and isolating said 39 kilodalton α protein from said host cells.

71. A method of producing a recombinant *Borrelia burgdorferi* 39 kilodalton β protein comprising culturing host cells according to claim 68, in a manner allowing expression of said 39 kilodalton β protein, and isolating said 39 kilodalton β protein from said host cells.

72. The proteins produced by the method of claim 69.

73. The protein produced by the method of claim 70.

74. The protein produced by the method of claim 71.

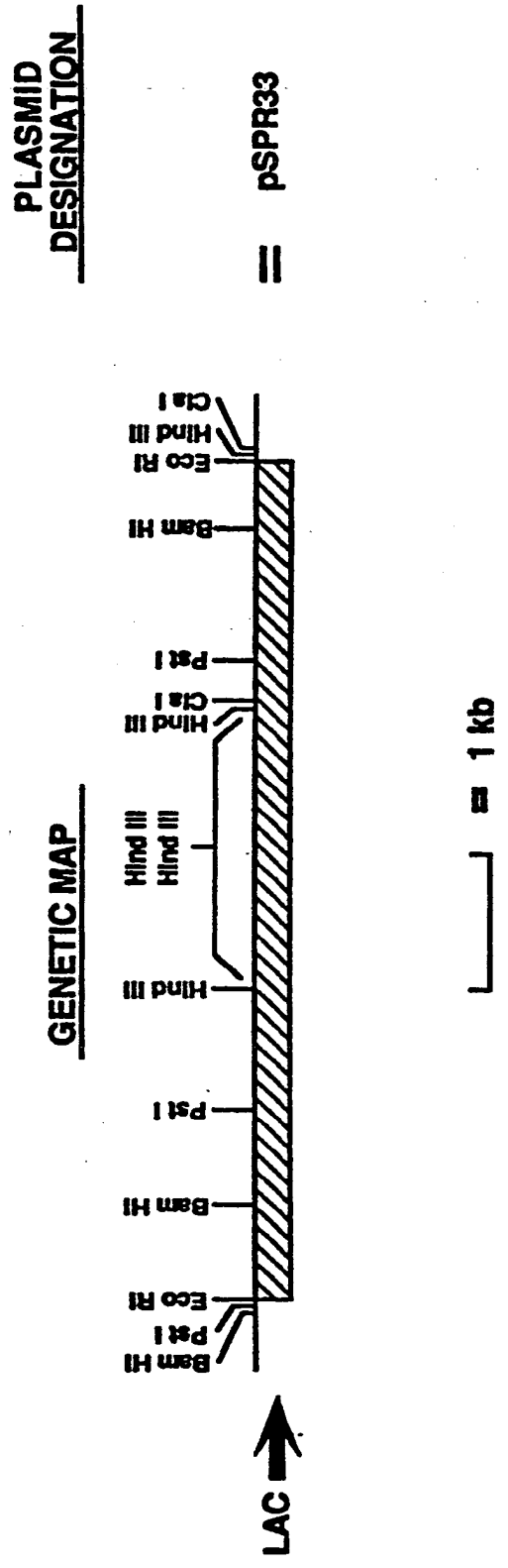
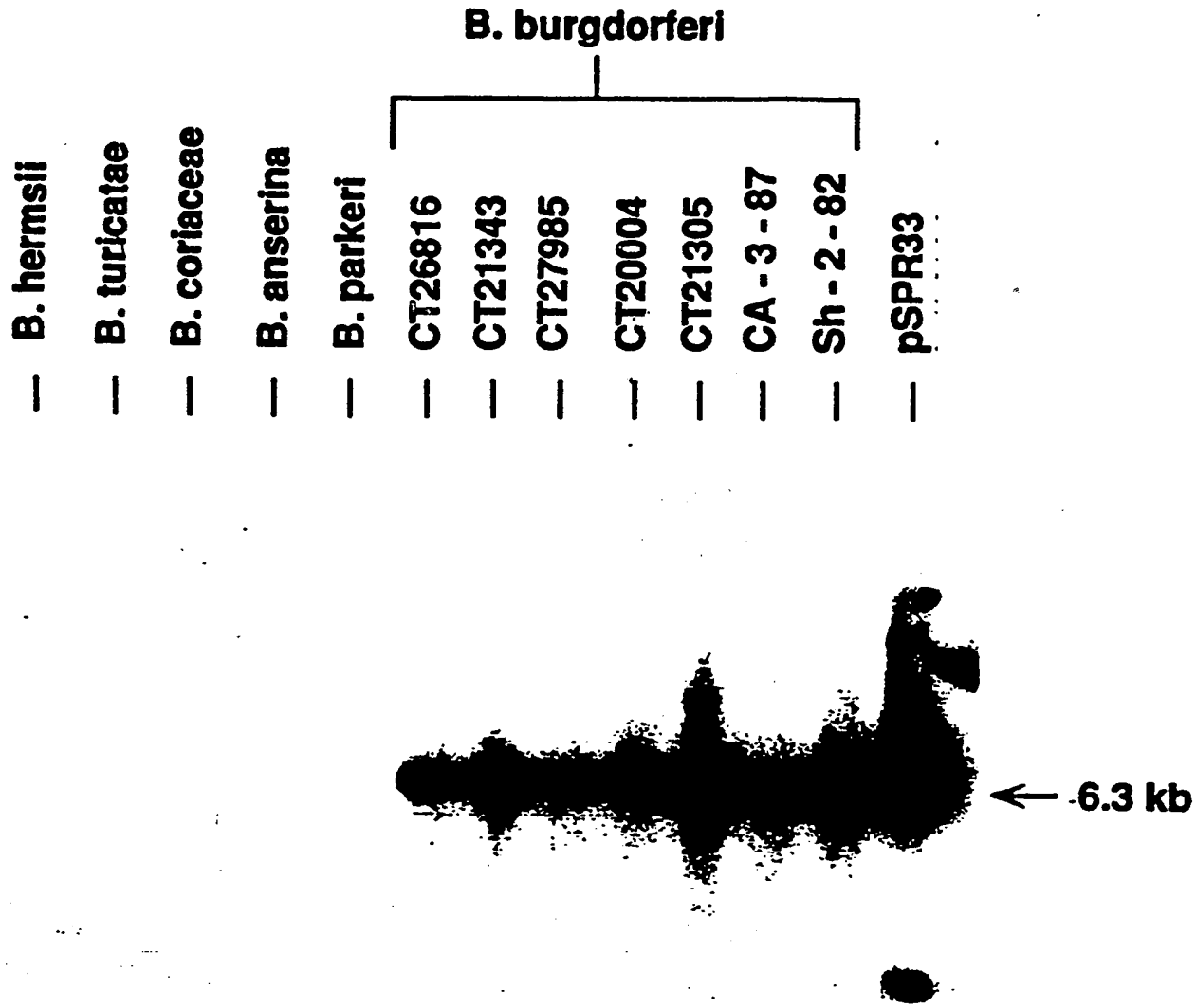

PLASMID DESIGNATION
pSPR33
GENETIC MAP
Cla I
Hind III
Eco RI
Bam HI
Pst I
Cla I
Hind III
Hind III
Hind III
Pst I
Bam HI
Eco RI
Pst I
Bam HI
LAC
1 kb

Fig. 1

Fig. 2


B. burgdorferi
B. hermsii
B. turicatae
B. coriaceae
B. anserina
B. parkeri
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CT21343
CT27985
CT20004
CT21305
CA-3-87
Sh-2-82
pSPR33
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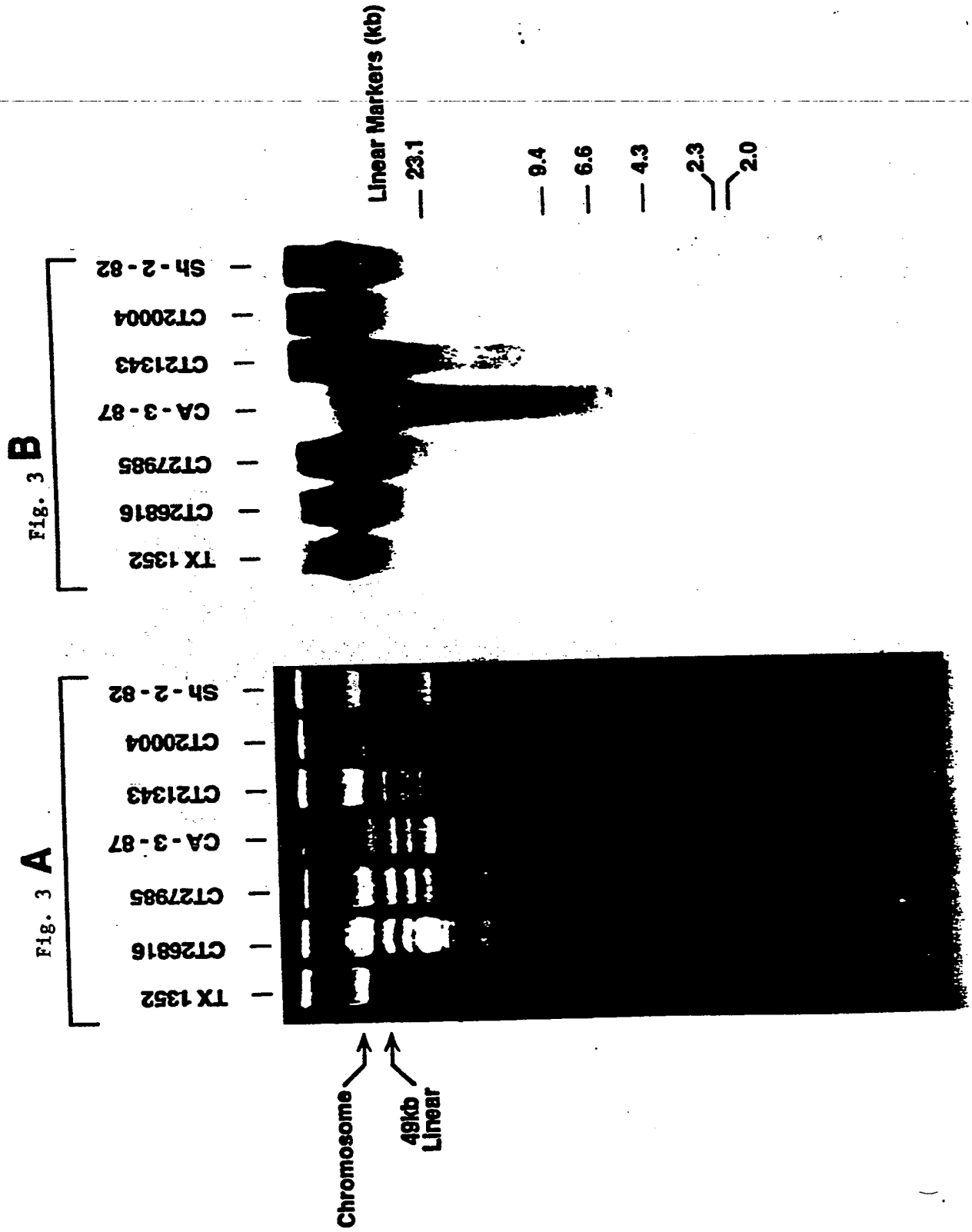
Linear Markers (kb)
23.1
9.4
6.6
4.3
2.3
2.0
Sh-2-82
CT20004
CT21343
CA-3-87
CT27985
CT26816
TX 1352
Fig. 3 B
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CT27985
CT26816
TX 1352
Fig. 3 A
Chromosome
49kb Linear

Fig. 4

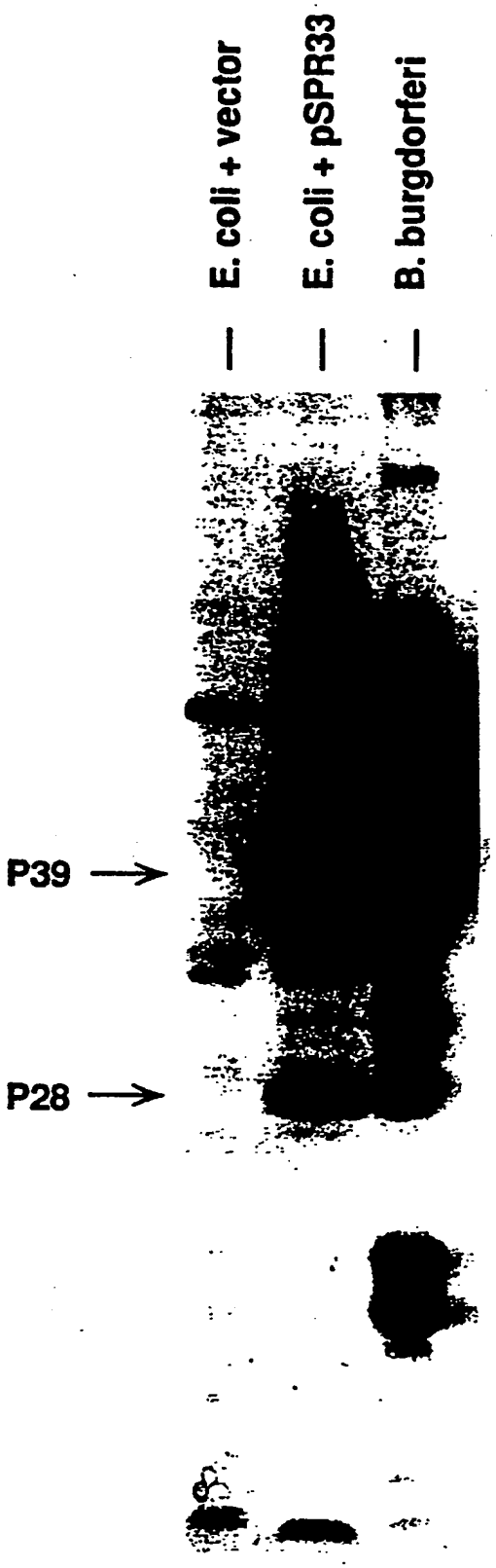


Fig. 5

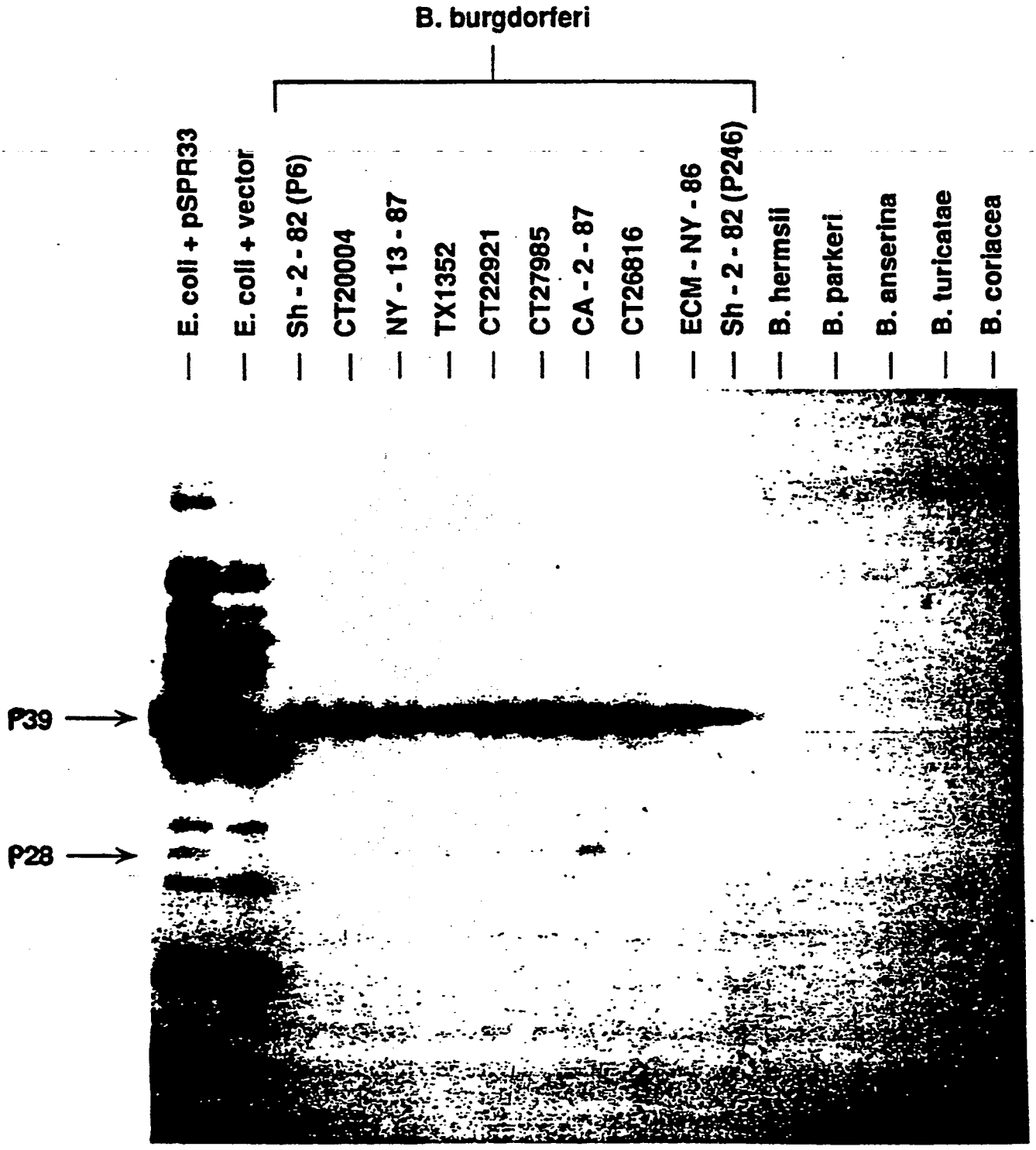

B. burgdorferi
E. coli + pSPR33
E. coli + vector
Sh - 2 - 82 (P6)
CT20004
NY - 13 - 87
TX1352
CT22921
CT27985
CA - 2 - 87
CT26816
ECM - NY - 86
Sh - 2 - 82 (P246)
B. hermsii
B. parkeri
B. anserina
B. turicatae
B. coriacea
P39
P28

Fig. 6

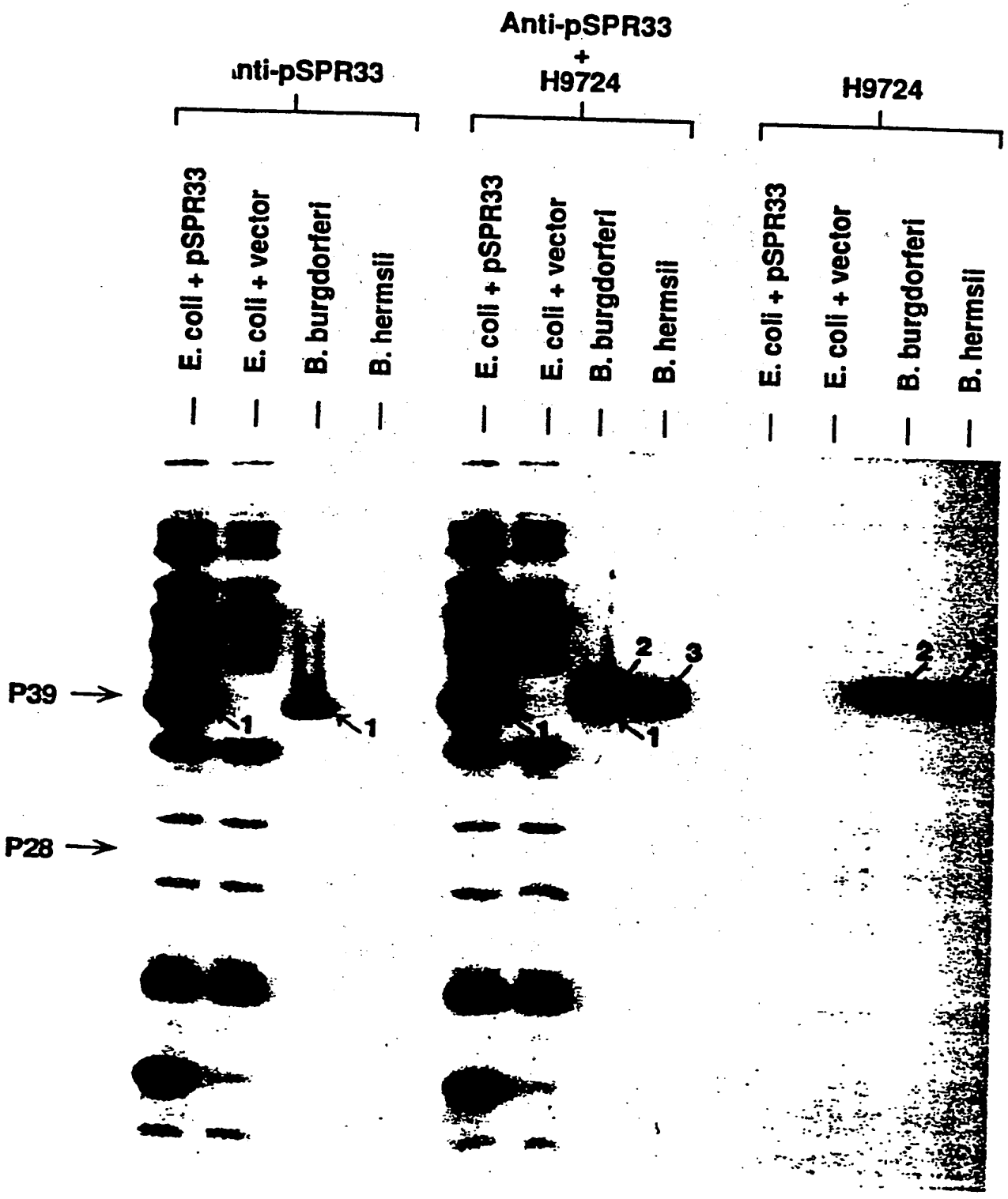


Fig. 7 (A)

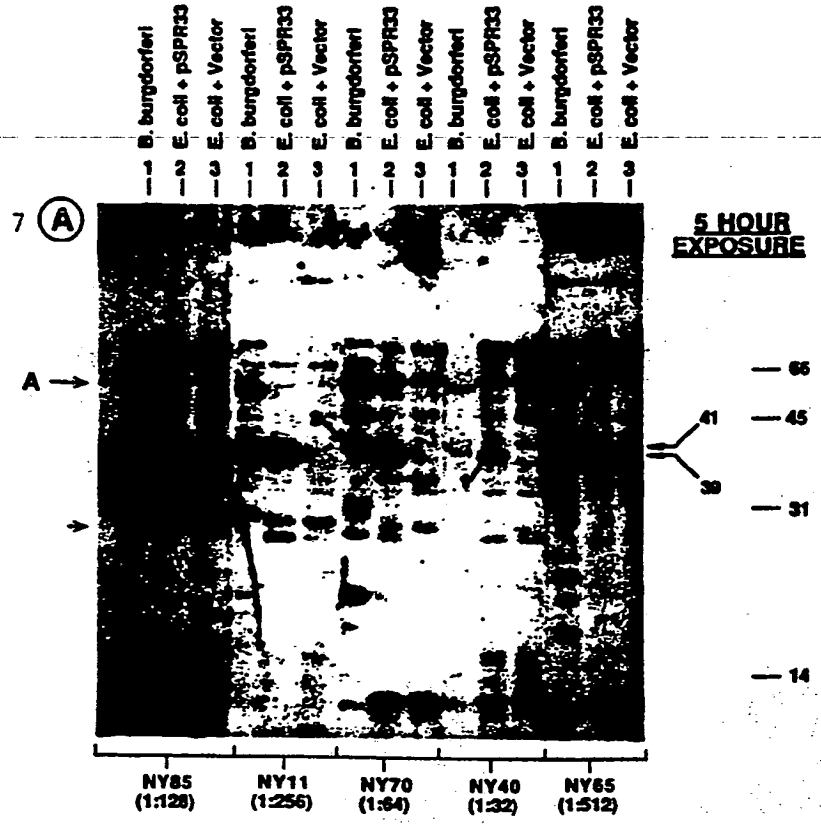
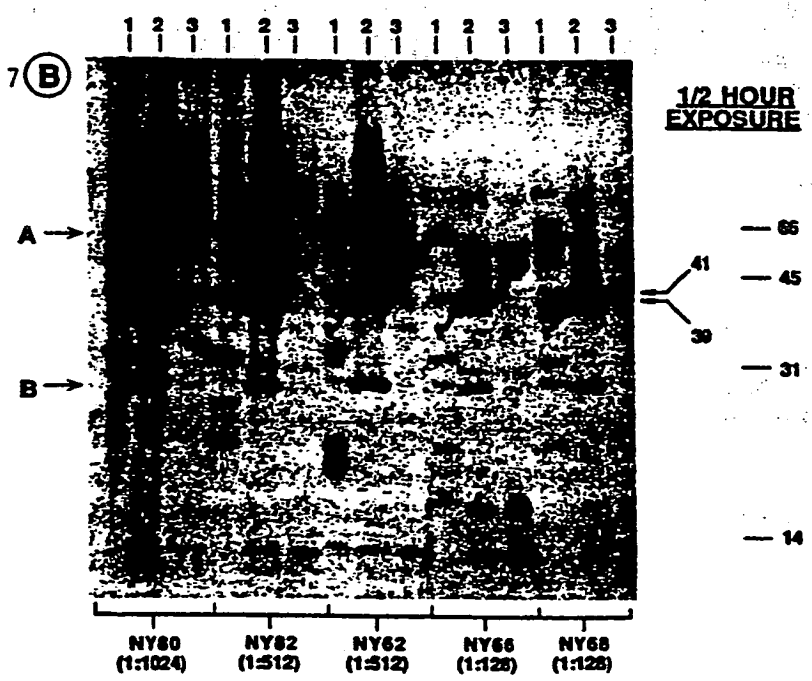


Fig. 7 (B)



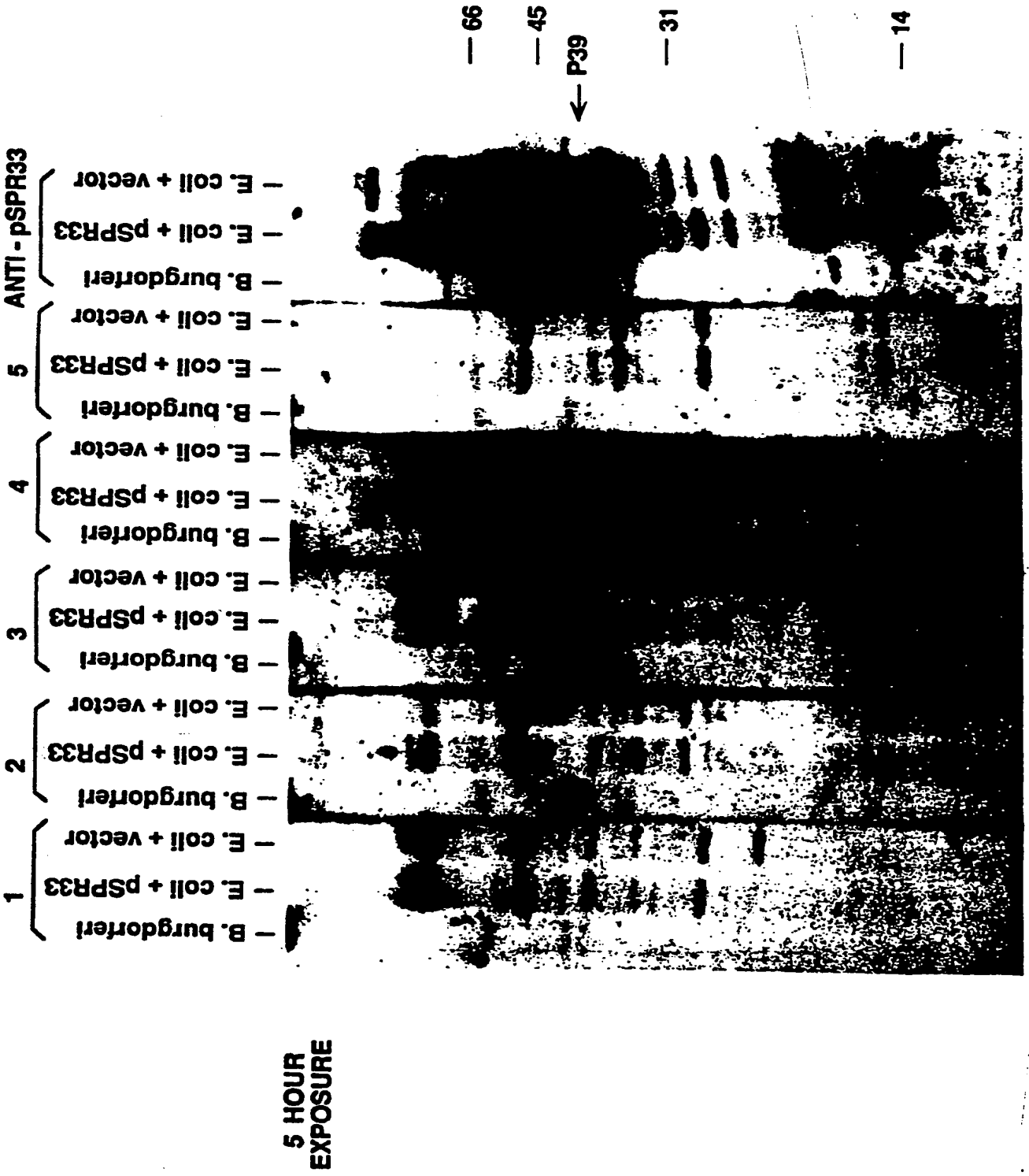

66
45
P39
31
14
ANTI - pSPR33
1
2
3
4
5
B. burgdorferi
E. coli + pSPR33
E. coli + vector
5 HOUR EXPOSURE

Fig. 8

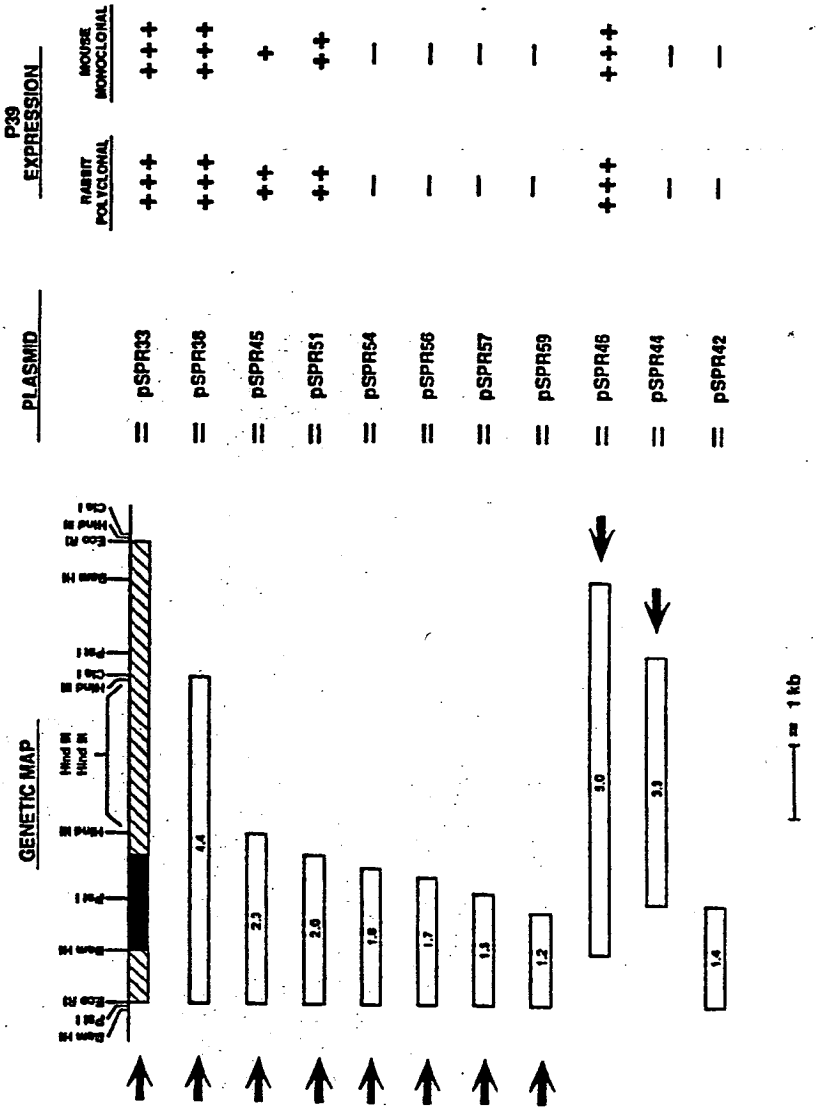

P39 EXPRESSION
MOUSE MONOCLONAL
RABBIT POLYCLONAL
PLASMID
pSPR33 +++ +++
pSPR36 +++ +++
pSPR45 + ++
pSPR51 ++ ++
pSPR54 - -
pSPR56 - -
pSPR57 - -
pSPR59 - -
pSPR46 +++ +++
pSPR44 - -
pSPR42 - -
GENETIC MAP
Hind III
4.4
2.3
2.0
1.8
1.7
1.5
1.2
3.0
3.3
1.4
1 kb

Fig. 9

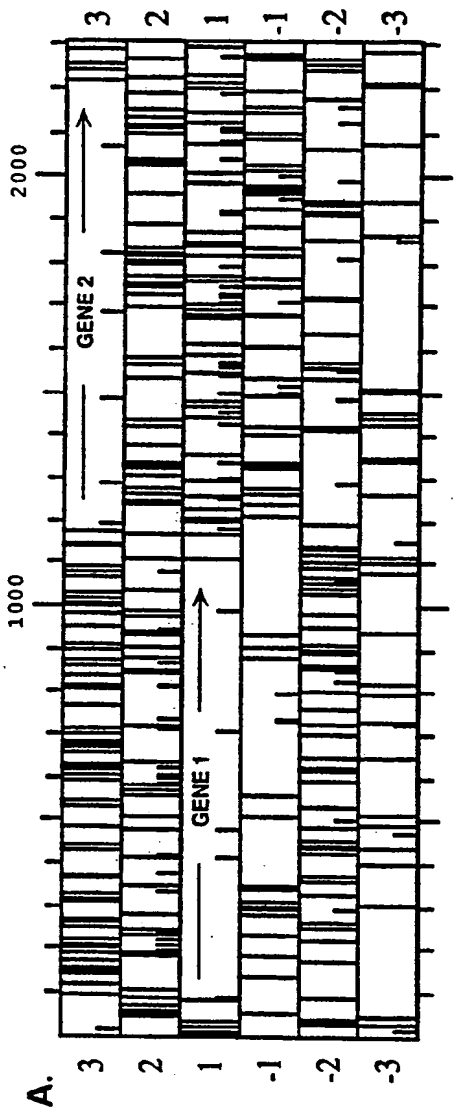


Fig. 10

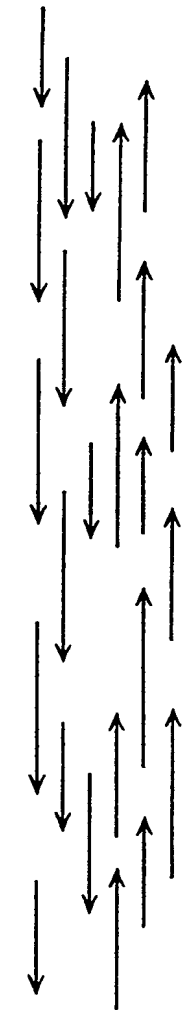


Fig. 10

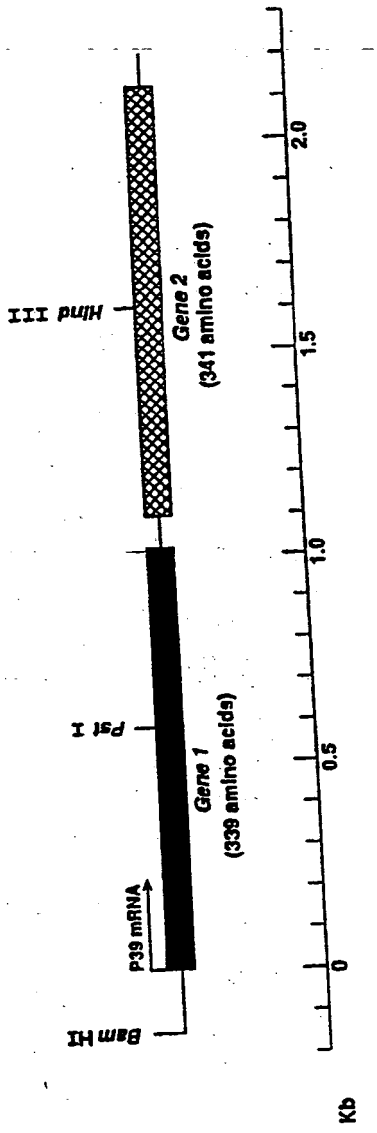

Hind III
Gene 2
(341 amino acids)
Pst I
Gene 1
(339 amino acids)
P39 mRNA
Bam HI
0
0.5
1.0
1.5
2.0
Kb

Fig. 11

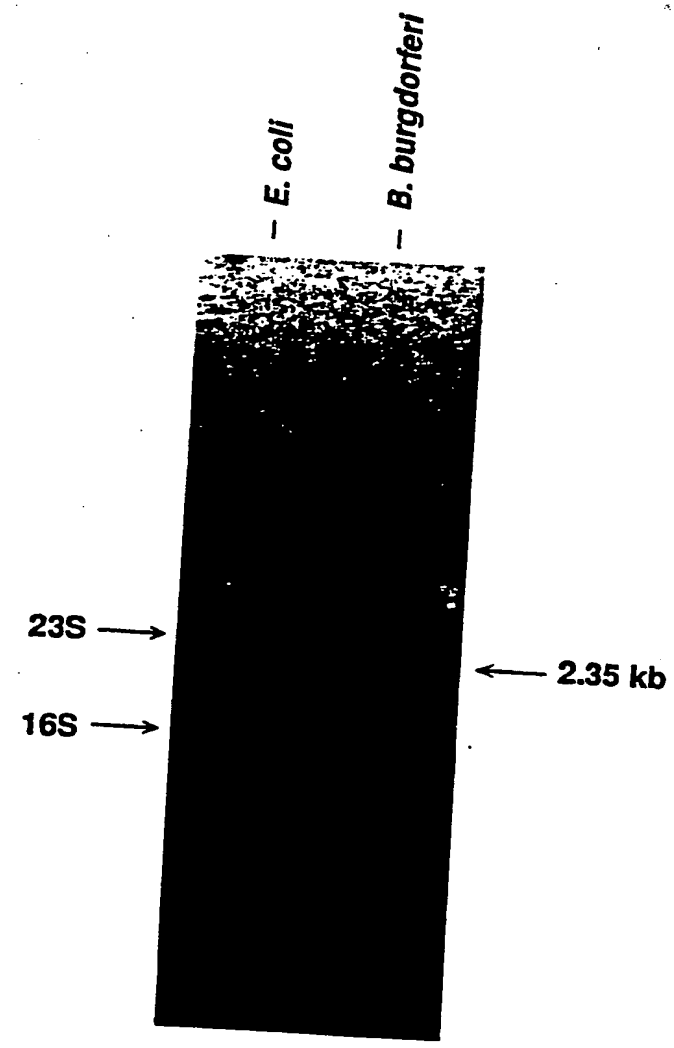


Fig. 12

Fig. 13

1				5				10				15				
Met	Asn	Lys	Ile	Leu	Leu	Leu	Ile	Leu	Leu	Glu	Ser	Ile	Val	Phe	Leu	Ser **Cys** Ser	GENE 1
Met	Arg	Ile	Val	Ile	Phe	Ile	Phe	Gly	Ile	Leu	**Leu**	Thr	Ser	**Cys**	Phe	Ser Arg Asn	GENE 2
Met	Lys	Lys	Tyr	Leu	Leu	Gly	Ile	Gly	Leu	Ile	Leu	Ala	**Leu**	Ile	Ala	**Cys** Lys Gln	OspA
Met	Arg	Leu	Leu	Ile	Gly	Phe	Ala	Leu	Ala	Leu	Ala	**Leu**	Ile	Gly	**Cys**	Ala Gln Lys	OspB

13/14

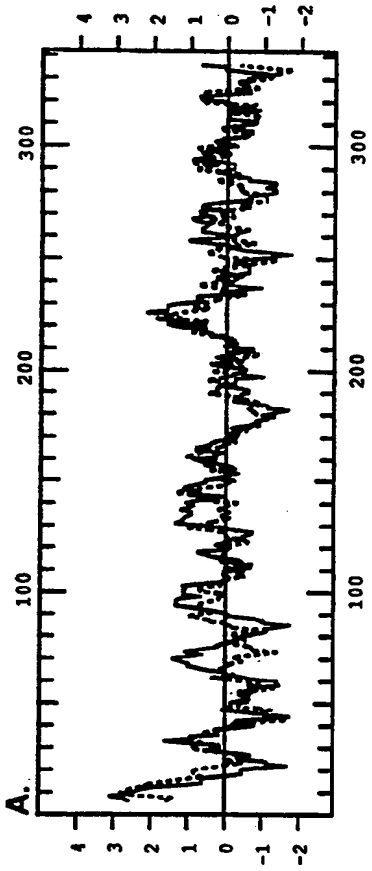


Fig. 14A

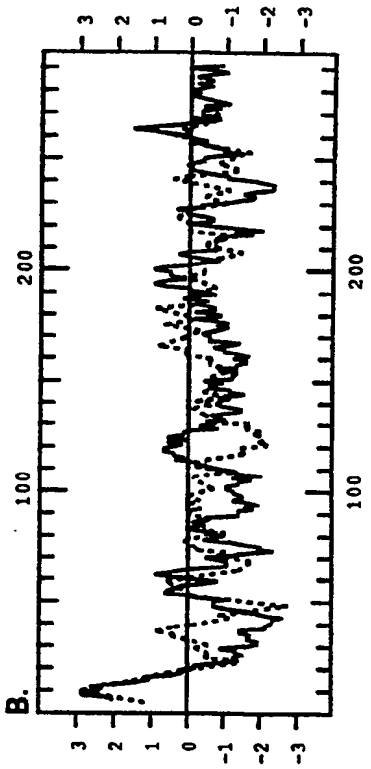


Fig. 14B

International Application No PCT/US 91/01500

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): A61K 39/00; C12Q 1/00; A01N 37/18; C07K 3/00

U.S.CL 424/85.8; 435/7.22; 514/2; 530/350

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
US: Cl.	435/7.22 69.1, 91, 172.1, 235, 240.2, 252.3, 320.1; 530/350; 424/85.8, 88, 93; 514/2

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

Chemical Abstract Data Base (cas) 1967-1991

Key words: Borrelia, burgdorferi, antigen, vaccine, assay.

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
	See Attached	

[10] Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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IV. CERTIFICATION

Date of the Actual Completion of the International Search

28 May 1991

Date of Mailing of this International Search Report

21 JUN 1991

International Searching Authority

ISA/US

Signature of Authorized Officer

Joan Ellis

Form PCT/ISA/210 (second sheet) (rev. 11-87)

Y,P	Journal of Clinical Microbiology, Volume 29, No. 2 , issued February 1991, W. J. Simpson, et al "Antibody to a 39-Kilodalton Borrelia burgdorferi Antigen (P39) as a Marker for Infection in Experimentally and Naturally Inoculated Animals", pages 236-243, see especially, the abstract.	1-74
A	Infection and Immunity, Volume 54, No. 1, issued October 1986, T. R. Hove, et al "Organization of Genes Encoding Two Outer Membrane Proteins of the Lyme Disease Agent Borrelia burdorferi Within a Single Transcriptional Unit", pages 207-212.	1-74
A	Journal of Clinical Microbiology, Volume 27, No. 8, issued August 1989, K. E. Hechemy, et al., "Fluoroimmunoassay Studies with Solubilized Antigens from Borrelia burgdorferi", pages 1854-1858.	49-59
A	Reviews of Infectious Diseases, Volume 11, Supplement 6, issued September-October 1989, A. G. Barbour, "The Molecular Biology of Borrelia", pages S1470-S1474.	1-74

CORRECTED VERSION *

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(54) Title: ANTIGENIC PROTEINS OF *BORRELIA BURGDORFERI*

(57) Abstract

The present invention relates to antigenic proteins specific to *Borrelia burgdorferi* which have a molecular weight of 28 kDa or 39 kDa as determined by SDS-PAGE and are reactive with Lyme borreliosis serum or fragments thereof and to the corresponding DNA. The proteins, especially the 39 kDa proteins (α and β) can be used to diagnose mammals previously or currently infected with the Lyme borreliosis causing agent.

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ANTIGENIC PROTEINS OF *BORRELIA BURGDORFERI*

FIELD OF THE INVENTION

The present invention relates to antigenic *Borrelia burgdorferi* proteins and their encoding DNA. In particular, the present invention relates to two 39 kilodalton (kDa) *Borrelia burgdorferi* proteins which react with Lyme borreliosis serum and a 28 kDa *Borrelia burgdorferi* protein which reacts with Lyme borreliosis serum.

BACKGROUND INFORMATION

Lyme borreliosis in humans is a multisystemic disorder caused by infection with the tick-borne spirochete, *Borrelia burgdorferi*, (Burgdorfer et al. 1982. Science 216:1317-1319; Johnson et al. 1984. Int. J. Syst. Bacteriol. 34:496-497; and Steere et al. 1983. N. Engl. J. Med. 308:733-740). Since the first epidemiological investigations of this disease in south-central Connecticut (Steere et al. 1977. Ann. Intern. Med. 86:685-698 and Steere et al. 1977. Arthritis. Rheum. 20:7-17), human cases of Lyme borreliosis have now been acquired in 43 states of the United States (Centers for Disease Control 1989, Lyme Disease - United States, 1987 and 1988. MMWR 38:668-672), five provinces of Canada, (Centers for Disease Control 1989, Lyme disease - Canada. MMWR 38:677-678), numerous countries throughout Europe and Asia (Ai et al. 1988. Ann. NY Acad. Sci. 539:302-313; Dekonenko et al. 1988. J. Infect. Dis. 158:748-753; and Schmid. 1985. Rev. Infect. Dis. 7:41-50), and possibly restricted foci in Australia (Stewart et al. 1982. Med. J. Australia 1:139) and Africa (Haberberger et al. 1989. Trans. R. Soc. Trop. Med. Hyg. 83:556 and Stanek et al. 1986. Zentralbl. Bakteriol. Mikrobio. Hyg. [A] 263:491-495). Between 1982-1988, reports of 13,825 cases of Lyme borreliosis were received by the Centers for Disease Control from all 50 states of the United States, (Centers for Disease Control 1989, Lyme Disease - United States, 1987 and 1988. MMWR 38:668-672), making this disease the most prevalent arthropod-borne infection in the country.

With the dramatic increase in awareness, prevalence, and geographical distribution of Lyme borreliosis, a tremendous new demand has been placed on clinical laboratories for serological confirmation of cases, (Magnarelli. 1989. J. Am. Med. Assoc. 262:3464-3465 and Schwartz et al. 1989. J. Am. Med. Assoc. 262:3431-3434) or to rule out this disease in differential diagnoses. However, many potential problems exist with the currently available serological tests for Lyme borreliosis, which may result in either false positive or false negative results (Magnarelli 1989. J. Am. Med. Assoc. 262:3464-3465). Some studies have focused on using flagellar protein of *B. burgdorferi* to increase the sensitivity of serological tests (Hansen et al. 1989. J. Clin. Microbiol 27:545-551 and Hansen et al. 1988. J. Clin. Microbiol 26:338-346) because earlier studies demonstrated that it appeared to be the 41 kilodalton (kDa) flagellar subunit (flagellin) of the spirochete that generated the earliest antibody response in infected humans (Barbour et al. 1983. J. Clin. Invest. 72:504-515; Coleman et al. 1987. J. Infect. Dis. 155:756-765; and Grodzicki et al. 1988. J. Infect. Dis. 157:790-797). One of two potential problems with using flagellar protein, however, is that flagella of other *Borrelia* species share epitopes common to the flagella of *B. burgdorferi* (Barbour et al. 1986. Infect. Immun. 52:549-544). Secondly, in most studies that have screened human sera by immunoblot analysis (Barbour. 1984. Yale J. Biol. Med. 57:581-586; Barbour et al. 1983. J. Clin. Invest. 72:504-515; Coleman et al. 1987. J. Infect. Dis. 155:756-765; Craft et al. 1986. J. Clin. Invest. 78:934-939; and Nadal et al. 1989. Pediatr. Res. 26:377-382), antibodies binding the protein with an apparent migration of 41 kDa have been assumed, but not proven, to be flagellin.

Thus, it is clear that a need exists for a method of detecting Lyme borreliosis disease in mammals. The present invention provides such a method.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a means for detecting mammals previously or presently infected with Lyme disease.

In one embodiment, the present invention relates to substantially pure forms of a *Borrelia burgdorferi* proteins which have molecular weights of about 39 kilodaltons and a protein which has a molecular weight of about 28 kilodaltons as determined by SDS-PAGE and which are reactive with Lyme borreliosis serum.

In another embodiment, the present invention relates to *Borrelia burgdorferi* proteins substantially free of proteins with which they are normally associated that have molecular weights of about 39 kilodaltons and a protein which has a molecular weight of about 28 kilodaltons as determined by SDS-PAGE and which are reactive with Lyme borreliosis serum.

In yet another embodiment, the present invention relates to a DNA fragment encoding all, or a unique portion, of the above described 39 kilodalton *Borrelia burgdorferi* proteins or the 28 kilodalton *Borrelia burgdorferi* protein.

In another embodiment, the present invention relates to a DNA fragment encoding all, or a unique portion, of one of the above described 39 kilodalton *Borrelia burgdorferi* proteins.

In a further embodiment, the present invention relates to a recombinant DNA molecule comprising a fragment of the above described DNA and a vector. The invention also relates to a host cell stably transformed with such a recombinant DNA molecule in a manner allowing expression of the *Borrelia burgdorferi* proteins encoded in the DNA fragment.

In another embodiment, the present invention relates to a method of producing recombinant *Borrelia burgdorferi* proteins of about 39 kilodaltons and a protein of about 28 kilodaltons and which are reactive with Lyme borreliosis serum which method comprises culturing host

cells expressing the proteins, in a manner allowing expression of the proteins, and isolating the proteins from the host cells.

In a further embodiment, the present invention relates to a purified form of an antibody specific for the above described 39 kilodalton *Borrelia burgdorferi* proteins or a unique fragment thereof or the above described 28 kilodalton *Borrelia burgdorferi* protein or a unique fragment thereof.

In another embodiment, the present invention relates to a vaccine for mammals against Lyme disease comprising all, or a unique portion, of the above described 39 kilodalton *Borrelia burgdorferi* proteins, each of the 39 kDa proteins or the above described 28 kilodalton protein *Borrelia burgdorferi* protein which are reactive with Lyme borreliosis serum, in an amount sufficient to induce immunization against Lyme disease, and a pharmaceutically acceptable carrier.

In a further embodiment, the present invention relates to a bioassay for the diagnosis of Lyme borreliosis disease comprising the steps of coating a surface with the 39 kDa proteins, each of the 39 kDa proteins or the 28 kDa protein of this invention (or antibodies specific therefor), contacting the surface with serum and detecting the presence or absence of a complex formed between the coated proteins (or coated antibodies) and antibodies specific therefor (or the target protein) in the serum.

In another embodiment, the present invention relates to a diagnostic kit comprising natural or recombinantly produced *Borrelia burgdorferi* 39 kDa proteins, each of the 39 kDa proteins or a 28 kDa protein and ancillary reagents suitable for use in detecting the presence of antibodies to the protein in a mammalian tissue sample.

In yet another embodiment, the present invention relates to a method of screening drugs for anti-Lyme borreliosis disease activity comprising contacting the

drug with cells contacted with *Borrelia burgdorferi* under conditions such that inhibition of anti-Lyme activity can be effected.

Various other objects and advantages of the present invention will become obvious from the drawings and the following description of the invention.

All publications mentioned herein are hereby incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows a genetic map of pSPR33. Spirochete DNA is denoted as a striped block and the arrow indicates the direction that the *Lac* promoter is transcribed. There were no restriction sites within the spirochete *Eco*RI fragment for *Acc*I, *Kpn*I, *Xba*I, *Xho*I or *Sma*I.

FIGURE 2 is an autoradiograph showing hybridization of ^{32}P-labeled insert DNA from pSPR33 with total DNA digested with *Eco*RI from 7 isolates of *B. burgdorferi* and 5 other *Borrelia* species. The right lane contained pSPR33 (pSPR33) digested with *Eco*RI. Linear molecular weight markers (Kb) are indicated on the right of the panel.

FIGURE 3 is an ethidium bromide stained gel of undigested total DNA from 7 isolates of *B. burgdorferi* (panel A) and an autoradiograph of the same gel after blotting to nitrocellulose and hybridization with the ^{32}P-labeled 6.3 Kb *Eco*RI fragment from pSPR33 (panel B). Note the strong hybridization signal associated with the chromosomal band.

FIGURE 4 shows immunoblot analysis of proteins expressed by pSPR33. Whole-cell lysates of *B. burgdorferi* strain Sh-2-82, *E. coli* carrying pSPR33 (*E. coli* + pSPR33) and *E. coli* carrying only vector (*E. coli* + vector) were immunoblotted with the human Lyme borreliosis serum used to screen the DNA library of *B. burgdorferi*.

FIGURE 5 demonstrates the specificity of P28 and P39 expression in *B. burgdorferi*. Whole-cell lysates of different *B. burgdorferi* strains (including low (P6) and

high (P246) *in vitro* passages of strain Sh-2-82) and isolates representing 5 additional *Borrelia* species were immunoblotted with anti-pSPR33 serum. Lysates of *E. coli* that express P28 and P39 (*E. coli* + pSPR33) and that do not (*E. coli* + vector) were also immunoblotted as positive and negative controls respectively. Not all of the 20 *B. burgdorferi* isolates tested are shown (see Table 1).

FIGURE 6 comprises the reactivity of anti-pSPR33 and monoclonal antibody H9724. Components in whole cell lysates of *E. coli* plus pSPR33, *E. coli* plus vector only, *B. burgdorferi* strain Sh-2-82 and *B.hermsii* strain FRG were separated by SDS-PAGE and were incubated with anti-pSPR33, anti-pSPR33 plus H9724, or H9724. P39 (arrow 1); 41 kDa flagellin from *B. burgdorferi* (arrow 2); 39 kDa flagellin from *B. hermsii* (arrow 3).

FIGURE 7 shows immunoblot analysis of 10 human Lyme borreliosis sera and their reactivity with P28 and P39. Whole-cell lysates of *B. burgdorferi* strain Sh-2-82 (lane 1), *E. coli* carrying pSPR33 (lane 2) and *E. coli* carrying only vector (lane 3) were immunoblotted with human Lyme borreliosis sera (NY). IFA Lyme borreliosis titers for each human serum are indicated below their designations. Autoradiographs exposed for 5 hr. (panel A) represented sera having weaker reactivity than those exposed for 1/2 hr. (panel B). Arrows denote P39 (arrow 1) and a 41 kDa antigen (arrow 2). Band B corresponds to the position of P28 and band A is an 58-65 kDa antigen that bound all sera that reacted with P39. Molecular mass markers (kDa) are indicated on the right of each panel.

FIGURE 8 shows immunoblot analysis of syphilitic sera. Whole-cell lysates of *B. burgdorferi* strain Sh-2-82, *E. coli* carrying pSPR33 and *E. coli* carrying only vector were immunoblotted with 5 syphilitic sera (1 to 5) or anti-pSPR33 (anti-pSPR33). Molecular mass markers are indicated on the right. Note absence of P39 in pSPR33 lanes reacted with syphilitic sera which contrasts with a strongly reactive 41 kDa antigen in three of the five *B. burgdorferi* lanes.

FIGURE 9 shows a restriction endonuclease map and expression data for the P39 locus of *Borrelia burgdorferi*. Subclones and deletion variants of plasmid pSPR33 (pSPR38, pSPR45, pSPR51, pSPR54, pSPR56, pSPR57, pSPR59, pSPR38, pSPR45, pSPR51, pSPR54, pSPR56, pSPR57, pSPR59, pSPR46, pSPR44 and pSPR42) are indicated as open bars.

FIGURES 10a and 10b show a map of the open reading frames of gene 1 and gene 2 encoding the P39 α and P39 β antigens, respectively, of *Borrelia burgdorferi*. Figure 10a shows the frames clear of termination sites (complete vertical lines). Figure 10b shows primer sites with overlapping sequences used to determine nucleotide sequences of both strands of DNA.

FIGURE 11 shows a genetic map of the P39 operon of *Borrelia burgdorferi* including the position of genes 1 and 2, the number of deduced amino acid, and the direction of transcription.

FIGURE 12 shows northern blot of *Borrelia burgdorferi* RNA probed with pSPR33 showing a 2.35 kb RNA transcript of the appropriate size for the single transcriptional unit for genes 1 and 2.

FIGURE 13 shows the deduced amino-terminal ends of P39 α (gene 1) and P39 β (gene 2), and the major outer surface proteins (Osp) A and B of *Borrelia burgdorferi*.

FIGURE 14 shows hydrophobicity plots of the deduced amino acid sequences of P39 α (dotted line) and P39 β (solid line) (Panel A) and OspA (dotted line) and OspB (solid line) (Panel B) of *Borrelia burgdorferi* (+ values show hydrophilic regions and - values show hydrophobic regions of the proteins).

DETAILED DESCRIPTION OF THE INVENTION

This invention relates, in part, to *Borrelia burgdorferi* antigenic proteins and their encoding DNA. A principle embodiment of this aspect of the present invention relates to three antigenic *Borrelia burgdorferi* proteins. Two proteins are characterized by a molecular weight of about 39 kDa (designated 39α and 39β) as determined by SDS-PAGE and reactivity with human Lyme

borreliosis serum. The third protein is characterized by a molecular weight of about 28 kDa as determined by SDS-PAGE and reactivity with human Lyme borreliosis serum. The present invention also relates to unique portions of the above proteins wherein a unique portion consists of at least 5 (or 6) amino acids.

The 39 kDa and 28 kDa proteins are substantially free of proteins with which they are normally associated. A substantially pure form of the proteins of the present invention can be obtained by one skilled in the art using standard methodologies for protein purification without undue experimentation. The present invention also relates to peptide fragments of the 39 kDa or 28 kDa protein. Alternatively, the proteins and peptides of the invention can be chemically synthesized using known methods.

The present invention also relates to a DNA fragment encoding all, or a unique portion, of the 39 kDa *B. burgdorferi* proteins or the 28 kDa *B. burgdorferi* protein of the present invention. A principle embodiment of this aspect of the invention relates to the 6.3 kilobase pair *Eco*RI fragment obtained from a DNA library of *B. burgdorferi* DNA which encodes the 39 kDa and 28 kDa antigenic proteins.

The present invention also relates to a DNA fragment encoating all, or a unique portion, of the 39 kDa α *B. burgdorferi* protein or the 39 kDa β *B. burgdorferi* protein.

The present invention further relates to a recombinant DNA molecule and to a host cell transformed therewith. Using standard methodology well known in the art, a recombinant DNA molecule comprising a vector and a DNA fragment encoding both the 39 kDa proteins of this invention, either of the 39 kDa proteins or the 28 kDa protein can be constructed using methods known in the art without undue experimentation. The DNA fragment can be isolated from *B. burgdorferi*, and it can take the form of a cDNA clone produced using methods well known to those skilled in the art or it can be produced by polymerase

chain reaction. Possible vectors for use in the present invention include, but are not limited to, λZAPII, pUC8 or preferably high frequency expression vectors such as pBluescript II SK, pNH8a. The host cell can be prokaryotic (such as bacterial), lower eukaryotic (such as fungal, including yeast) or higher eukaryotic (such as mammalian).

The present invention further relates to antibodies specific for the 39 kDa *B. burgdorferi* proteins or the 28 kDa protein of the present invention. One skilled in the art using standard methodology can raise monoclonal antibodies and polyclonal antibodies to the 39 kDa proteins or the 28 kDa protein, or a unique portion thereof. This is exemplified by the anti-pSPR33 rabbit antiserum (see Example 2 below).

The present invention also relates to a vaccine for use in mammals against Lyme borreliosis disease. In one embodiment of this aspect of this invention, as is customary for vaccines, the 39 kDa proteins, either of the 39 kDa proteins or the 28 kDa protein of the present invention can be delivered to a mammal in a pharmacologically acceptable vehicle. As one skilled in the art will understand, it is not necessary to use the entire protein. A unique portion of the protein (for example, a synthetic polypeptide corresponding to a portion of the 39 or 28 kDa proteins) can be used. Vaccines of the present invention can include effective amounts of immunological adjuvants known to enhance an immune response. The protein or polypeptide is present in the vaccine in an amount sufficient to induce an immune response against the antigenic protein and thus to protect against Lyme borreliosis infection. Protective antibodies are usually best elicited by a series of 2-3 doses given about 2 to 3 weeks apart. The series can be repeated when circulating antibodies concentration in the patient drops.

The present invention further relates to diagnostic assays for use in human and veterinary medicine. For diagnosis of Lyme borreliosis disease, the

presence of antibodies to the 39 kDa proteins or the presence of the 28 kDa proteins in mammalian serum is determined. Many types of tests, as one skilled in the art will recognized, can be used for detection. Such tests include, but are not limited to, IFA, RIA, RIST, ELISA, aggulination and hemagglutination. The diagnostic assays can be performed using standard protocols such as those described by Magnarelli et al. 1984. J. Clin. Microbiol. 20:181-184; Craft et al. 1984. J. Infect. Dis. 149:789-795; Enguall et al. 1971. Immunochemistry 8:871-874; and Russell et al. 1984. J. Infect. Dis. 149:465-470.

Specifically, a diagnostic assay of the present invention can be constructed by coating on a surface (ie. a solid support) for example, a microtitration plate or a membrane (eg. nitrocellulose membrane), all or a unique portion of the 39 kDa proteins (natural or synthetic), either of the 39 kDa proteins (natural or synthetic) or the 28 kDa protein (natural or synthetic) and contacting with the serum from a patient suspected of having Lyme borreliosis disease. The presence of a resulting complex formed between the surface and antibodies specific therefore in the serum can be detected by any of the known methods common in art, such as fluorescent antibody spectroscopy or colorimetry.

In another embodiment of the diagnostic assay of the present invention, all or a unique portion of the 39 kDa proteins, either of the 39 kDa proteins or the 28 kDa protein is bound to an inert particle of, for example, bentonite or polystyrene latex. The particles are mixed with serum from a patient in, for example, a well of a plastic agglutination tray. The presence or absence of antibodies in the patient's serum is determined by observing the settling pattern of the particles in the well.

In a further embodiment of the diagnostic assay of the present invention, the presence or absence of the 39 kDa proteins, or the 28 kDa protein in a serum sample is

detected. Antibodies specific for the 39 kDa proteins, either of the 39 kDa proteins or the 28 kDa protein or a unique portion thereof can be coated on to a solid surface such as a plastic and contacted with the serum sample. After washing, the presence or absence of the protein from the serum bound to the fixed antibodies is detected by addition of a labeled (e.g. fluorescently labeled) antibody specific for the 39 (or 28) kDa proteins.

One skilled in the art will appreciate that the invention includes the use of competition type assays in detecting in a sample the antigens and antibodies to which this invention relates.

The present invention further relates to screening for anti-Lyme borreliosis disease drugs. In one embodiment potential anti-Lyme borreliosis disease drugs are tested for their ability to inhibit expression of the 39 kDa proteins or the 28 kDa protein in cells contacted with the *B. burgdorferi*. The presence or absence of the 39 kDa proteins or 28 kDa protein in exposed cells treated with test drugs can be determined by any of the standard diagnostic assays mentioned above.

The present invention further relates to DNA fragments containing the nucleotide sequence as shown in Seq. Id No. 1-3, or mutants thereof, to recombinant molecules containing the DNA fragments and host cells transformed with the recombinant molecules. Using standard methodology well known in the art, a recombinant DNA molecule comprising a vector and the DNA fragments of this invention can be constructed using methods known in the art without undue experimentation. The DNA fragments can be isolated from *B. burgdorferi* or can be produced by a polymerase chain reaction. Possible vectors for use in the present invention include but are not limited to, pUC, pBluescript or pBR322. The host cell can be prokaryotic (such as bacterial), lower eukaryotic (such as fungal, including yeast) or higher eukaryotic (such as mammalian).

The present invention further relates to methods

of producing recombinant *Borrelia burgdorferi* 39 kDa and 28 kDa proteins comprising culturing the aforementioned host cells in a manner allowing expression of the proteins and isolating the proteins from the host cells. Methodology utilize to produce recombinant *B. burgdorferi* proteins are well within the skill of an ordinary artisan.

EXAMPLES

The following organisms and materials were used throughout the Examples.

Bacterial strains. *B. burgdorferi* strains used (See Table 1 below) have been previously described or were kindly provided by Dr. John Anderson (Connecticut Agriculture Experiment Station, New Haven, Conn.), Dr. Alan MacDonald (Southampton Hospital, Long Island, N.Y.), and Ms. Glenna Teltow and Ms. Julie Rawlings (Medical Entomology Section, Bureau of Laboratories, Texas Department of Health, Austin, Tex.). The five strains representing *B. hermsii* (HS1), *B. coriaceae* (Co53), *B. parkeri*, *B. turicatae* and *B. anserina* have been described previously (Schwan et al. 1989. J. Clin. Microbiol. 27:1734-1738). Borrelia organisms were cultured at 32°C in BSK-II medium as previously described (Barbour. 1984. Yale J. Biol. Med. 57:581-586).

TABLE 1. Summary of *Borrelia burgdorferi* strains used in this study all of which expressed P28 and P39.

Strain designation	*In vitro*° passages (H)igh/(L)ow	Biological* source	Geographical* source (year isolated)	Obtained from (reference)
Sh-2-82	(P6) L	Id	New York (1982)	Schwan et al. (1)
Sh-2-82	(P246) H	Id	New York (1982)	Schwan et al. (1)
B31	H	Id	New York (1982)	Schwan et al. (1)
CA-2-87	L	Ip	California (1987)	Schwan et al. (1)

	CA-3-87 L	Ip	California (1987)	Schwan et al. (1)
	NY-1-86 L	H	New York (1986)	Schwan et al. (1)
	ECM-NY-86 L	H	New York (1986)	Schwan et al. (1)
	NY-6-86 L	H	New York (1982)	MacDonald
	NY-13-86 L	H	New York (1982)	MacDonald
	CT20004 L	Ir	France (1985)	Anderson
	CT22921 L	Rp	New York (1986)	Anderson
	CT26816 L	Rm	Rhode Island (1985)	Anderson
	CT19678 L	Rp	New York (1986)	Anderson
	CT21343 L	Rp	Wisconsin (1986)	Anderson
	CT21305 L	Rp	Connecticut (1986)	Anderson
	CT21721 L	Id	Wisconsin (1986)	Anderson
	CT27985 L	Id	Connecticut (1988)	Anderson
	TX1352 H	Aa	Texas (1989)	Rawlings
	PE92 H	D	Texas (1989)	Rawlings
	BR4-3028 H	H	Texas (1989)	Rawlings

*Tick = *Ixodes dammini* (Id); tick = *I. pacificus* (Ip); tick = *I. ricinus* (Ir); tick = *Amblyomma americanum* (Aa); human (H); rodent = *Peromyscus leucopus* (Rp); rodent = *Microtus* (Rm); dog = (D).
°Strains passed for ≤ 10 passages (L); strains passed for ≥ 20 passages (H).
*USA state or country.
1= Schwan et al. 1989. J. Clin. Microbiol. 27:1734,1738.

Human syphilitic sera were kindly provided by Dr. Wayne Hogefre and Ms. Jane Markley (Hillcrest Biologicals, Cypress, Calif.), amyotrophic lateral sclerosis (ALS) sera were provided by Dr. Jeffrey Smith (Mount Sinai Medical Center, ALS Clinic, New York, NY.) and Dr. Alan MacDonald (Southampton Hospital, Long Island, N.Y.), and relapsing fever sera were collected from patients from Oregon and Washington. Normal sera were obtained from staff and laboratory personnel at Rocky Mountain Laboratories. Human Lyme borreliosis sera were provided by Dr. Alan MacDonald and were collected from patients clinically diagnosed with Lyme borreliosis from Long Island, New York.

Escherichia coli carrying the plasmid pSPR33 (see below) were deposited on February 28, 1990 at the American Type Culture Collection 12301 Parklawn Drive, Rockville, Maryland 20852. The accession number of the organism is 68243. The deposits shall be viably maintaining, replacing it if it becomes non-viable, for the life of the patent, for a period of 30 years from the date of the deposit or for five years from the last date of request or sample of the deposit, whichever is longer and made available to the public upon issuance of a patent from this application, without restriction, and in accordance with the provisions of the law. The Commissioner of Patents and Trademarks, upon request shall have access to the deposit.

Example 1. Cloning and Genetic Analysis of *Borrelia* DNA

To identify *B. burgdorferi* proteins that induce an antibody response during the course of an infection, a DNA library of *B. burgdorferi* containing *Eco*RI fragments was constructed in *E. coli* with the λ expression vector λZAPII.

Total DNA was purified from 500 ml stationary phase borrelial cultures by a modification as previously described (Barbour. 1988. J. Clin. Microbiol. 26:475-478). Cells were recovered by centrifugation, washed in 20 ml of PBS plus 5 mM $MgCl_3$ and resuspended in 2.4 ml TES (50 mM Tris, pH 8.0; 50 mM EDTA, 15% (w/v) sucrose). Lysozyme was added to a final concentration of 1 mg/ml and then the cell suspension was left on ice for 10 min. Cells were lysed by adding 3 ml TES plus 1% (v/v) sodium deoxycholate and gently mixed for 10 min. at room temperature. Proteinase K (1 mg) was then added and the sample was incubated at 37°C for 1 hr. The DNA suspension was then extracted twice with 1 volume of phenol-chloroform (1:1 (v/v)) and once with chloroform-isoamyl alcohol (24:1 (v/v)). The DNA was ethanol precipitated, washed twice with 70% ethanol and resuspended to a final concentration of 1 mg/ml in TE (10 mM Tris, pH 7.6; 1 mM EDTA).

Total DNA (1 μg) from *B. burgdorferi* strain Sh-2-

82 was digested with *Eco*RI, ligated to the dephosphorylated arms of the expression vector λZAPII (Stratagene, La Jolla, Calif.) and packaged according to the manufacturer's directions.

The library was screened for *Borrelia* by immunoblot with a convalescent serum from a human Lyme borreliosis patient from Long Island, New York (1:100) following absorption of plaque proteins to nitrocellulose filters (Maniatis et al. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). After blocking for 1 hr. at 25°C in TSE-Tween (50 mM Tris, pH 7.4; 150 mM NaCl; 5 mM EDTA; 0.05% Tween 20), filters were incubated with serum diluted in TSE-Tween with gentle rocking at 25°C for 1 hr. They were then washed for 1 hr. with four changes of TSE-Tween and the bound antibody was detected by incubating the filters with ^{125}I-labeled protein A (500,000 cpm/ml) for 1 hr. with rocking. Each filter was then washed four times for 15 min. each with TSE-Tween, dried and autoradiographed with Kodak X-AR5 film.

Positive clones were detected at a frequency of 5%. One recombinant plaque that reacted with human serum was plaque purified and the phagemid carrying the *Borrelia* DNA was excised from the λ sequences with the aid of the helper phage R407 according to the suppliers directions (Stratagene). Excision of the cloned fragment from the purified phage produced the phagemid portion containing a 6.3 kilobase (Kb) *Eco*RI fragment, designated plasmid pSPR33 (Fig. 1). The fragment was isolated from an agarose gel, radiolabeled and shown to hybridize with a similar sized fragment in *Eco*RI digested total DNA from all six North American and one European *B. Burgdorferi* isolates (Fig. 2).

Recombinant plasmid pPSR33 was isolated from *E. coli* for mapping studies from 500 ml cultures and purified as previously described (Simpson et al. 1987. Infect. Immun. 55:2448-2455), except two consecutive dye-buoyant density gradients were preformed (Plasterk et al. 1985.

Nature 318:257-263) in a Beckman VTi80 rotary at 70,000 rpm for 4 hr at 18 C. The supercoiled circular plasmid portion was diluted with two volumes of water after the removal of the ethidium bromide and then ethanol precipitated. The plasmid DNA was then resuspended in a minimal volume of TE. Mini-plasmid preparations (Maniatis et al. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York) of positive clones were examined by agarose gel electrophoresis after their digestion with *Eco*RI to determine the insert size.

Southern blot analysis of undigested DNA from seven similar isolates, indicated that the 6.3 Kb fragment hybridized strongly with chromosomal DNA (Fig. 3). Undigested total DNA was electrophoresed in 0.4% agarose gels (12 v for 16 hrs). Southern blot procedures including the transfer of DNA from agarose gels to nitrocellose, high stringency hybridization (which permitted 10% basepair mismatch), and autoradiography were as previously described (Spanier et al. 1983. Virology 130:514-522) except that the prehybridization and hybridization buffers and temperatures were as described by Schwan et al. (Schwan et al. 1989. J. Clin. Microbiol. 27:1734-1738).

The DNA probe was recovered from agarose gels using Gene Clean (BIO 101, Inc., La Jolla, Calif.) and labeled with [α-^{32}P]dCTP (3,000 Ci/mmol) by nick translation according to the directions of the manufacturer (Nick Translation Kit, Bethesda Research Laboratories, Gaithersburg, Md.). The probe was boiled for 4 min. and quenched on ice immediately before adding to the hybridization buffer.

Restriction endonucleases were purchased from Boehringer Mannheim Biochemicals, Indianapolis, Ind., and digestions were performed as recommended by the manufacturer.

The smeared band in agarose-gels that contained heterogenous fragmented DNA and migrated slightly slower

than the 49 Kb linear plasmid from strain Sh-2-82 was assumed to be chromosomal DNA. Total DNA from 5 additional *Borrelia* species including *B. hermsii, B. parkeri, B. anserina, B. turicatae,* and *B. coriaceae* did not hybridize to the 6.3 Kb fragment (Fig. 2). These data indicate that the pSPR33 insert sequences are chromosomally located and are specific to *B. burgdorferi*.

Example 2. Immunoblot Analysis of Cloned *B. burgdorferi* Proteins

To identify the specific proteins encoded by pSPR33 that reacted with the human serum used to screen the library, whole-cell lysates of *E. coli* carrying pSPR33 were analyzed by SDS-PAGE and immunoblot.

Rabbit serum prepared against whole-cell lysates of *E. coli* carrying either pSPR33 (anti-pSPR33) or the vector pBluescript SK (anti-*E. coli*) were prepared as follows. Bacterial cells recovered from 16 hr cultures, were washed once and resuspended in phosphate buffered saline (PBS) to a final concentration of 10^8 cells/ml. The cells were killed by incubating for 30 min. at 56°C and disrupted by sonification on ice (2 min. at an output of 4; Branson Sonifier-Cell Disrupter 185). New Zealand white rabbits were immunized (without adjuvant) intramuscularly with 1.5 ml of the cell sonicate and boosted with the same immunogen at 21 and 42 days after the primary immunization. Sera were collected every 2 weeks thereafter for 4 months, pooled, and 5 ml aliquots absorbed with *E. coli* strain XL1-blue cells (Stratagene) collected from 500 ml cultures and incubated with rotation at 37°C for 4 hr. The bacteria were removed by centrifugation in a VTi80 rotor at 40,000 rpm for 30 min. This process was repeated twice, and absorbed sera were then filtered through a sterile 0.22 μm filter (Millipore Corp., Medford, Mass.) and stored at -20°C. Anti-pSPR33 and anti-*E. coli* sera was used at a dilution of 1:500 and 1:50 respectively. The monoclonal antibodies H5332 (Barbour et al. 1983. Infect. Immun. 41:795-804), H5TS (Barbour et al. 1984. Infect. Immun. 45:94-100), and

H9724 (Barbour et al. 1986. Infect. Immun. 52:549-554) were used at a dilution of 1:100.

IFA titers of Lyme borreliosis and relapsing fever sera were determined as previously described (Burgdorfer et al. 1982. Science 216:1317-1319). *B. burgdorferi* strain B31 and *B. hermsii* strain HS1 respectively were used as the antigens in the IFA tests.

Immunoblot analysis of whole-cell lysates were performed essentially as previously described (Schwan et al. 1989. Infect. Immun. 57:3445-3451) except cells were prepared as follows. Cells were recovered from liquid cultures by centrifugation (8,000 xg for 5 min), and resuspended in PBS to give an optical density of 0.2 at 600 nm. Cells from 2 ml of this suspension were recovered by centrifugation and resuspended in 100 µl of distilled water and 50 µl of sample buffer (0.2 M Tris, pH 6.8; 30% (v/v) glycerol; 3% (w/v) SDS; 0.002% (w/v) bromophenol blue). Samples were then boiled for 4 min. and 20 µl loaded onto a 12.5% SDS-PAGE gel. Gel electrophoresis, immunoblotting and detection of bound antibody, using ^{125}I protein A, have been described (Schwan et al. 1989. Infect. Immun. 57:3445-3451).

A 28 kDa (P28) and a 39 kDa (P39) antigen in the pSPR33 immunoblot profile, were the most immunoreactive antigens that were not detected in lysates of *E. coli* cells carrying only the vector (Fig. 4). Antisera raised to whole-cell lysates of *E. coli* carrying only the vector (anti-*E. coli* serum) did not react with P28 or P39 at a dilution of 1:50. These two proteins, therefore, are antigenically unrelated to native *E. coli* components and appear to be encoded by the cloned *Borrelia* sequences. P28 and P39 could not be resolved in SDS-PAGE gels stained with either Coomassie blue or silver nitrate because they co-migrate with other, more abundant *E. coli* proteins.

Similar sized proteins to P28 and P39 were detected by immunoblot (Fig. 4) in cell lysates of *B. burgdorferi* strain Sh-2-82, suggesting P28 and P39 are expressed by this strain. To determine if the 28 and 39

kDa *Borrelia* proteins seen in whole cell lysates were identical to the gene products P28 and P39 respectively, antiserum generated to cells carrying pSPR33 (anti-pSPR33 serum) was incubated with Western blotted whole-cell lysates of 1 European and 19 North American *B. burgdorferi* isolates, and compared to a lysate of *E. coli* producing P28 and P39 (Fig. 5). All of the 20 *Borrelia* isolates expressed a 39 kDa protein that co-migrated with P39. A 28 kDa protein was also detected, but considerably less antibody bound this protein than that which bound P39. P39 produced by pSPR33 also reacted with sera from five white-footed mice *(Peromyscus leucopus)* experimentally infected with *B. burgdorferi* strain Sh-2-82, but did not react with the preimmune sera from these animals, or with sera from mice infected only with *E. coli*. Other species of *Borrelia* did not produce detectable amounts of P28, P39 or any other antigenically related proteins under the conditions employed (Fig. 5). Extended exposure (> 24 hours) of autoradiographs revealed weak bands with molecular weights other than 28 kDa and 39 kDa in all *Borrelia* profiles, but these are attributed to non-specific binding. Data, including the fact that DNA from other species of *Borrelia* lacked sequences with close identity to those that encode P28 and P39 (Fig. 2), show that P28 and P39 are proteins specific to *B. burgdorferi*. Furthermore, anti-pSPR33 did not react with the *B. burgdorferi* antigens Osp A (31 kDa), Osp B (34 kDa) or the 41 kDa flagellin, suggesting that these proteins are antigenically unrelated to P28 and P39 (Fig. 5).

To confirm this, it was shown that the monoclonal antibodies H5332, H5TS and H9724 (Fig. 6), which bind specifically to Osp. A (Barbour et al. 1983. Infect. Immun. 41:795-804), Osp B (Barbour et al. 1984. Infect. Immun. 45:94-100) and the flagellin (Barbour et al. 1986. Infect. Immun. 52:549-554) respectively, did not bind to P28 or P39 produced by either pSPR33 or strain Sh-2-82. The specificity of monoclonal antibody H9724 for *Borrelia* flagellin is evident in Figure 6, as this monoclonal only

bound a 41 kDa band in the *B. burgdorferi* profile and a 39 kDa band, which corresponds to its flagellin (Barbour et al. 1986. Infect. Immun. 52:549-554), in the *B. hermsii* profile. Furthermore, using electron microscopy and colloidal gold staining, monoclonal antibody H9724 bound to endoflagellin from *B. burgdorferi* whereas anti-pSPR33 did not.

Example 3. Immunoreactivity of Lyme Borreliosis Sera with Cloned Borrelia Proteins

To test the possibility that P28 and P39 are immunodominant proteins, ninety-four human sera collected from patients clinically diagnosed as having Lyme borreliosis were tested for reactivity with cloned P28 and P39 at a dilution of 1:100. Whole-cell lysates were electrophoresed in SDS-PAGE gels and Western blotted as previously described in the above Examples. The nitrocellulose was cut into equal strips (5 per gel) such that each strip contained lanes for *E. coli* carrying pSPR33, *E. coli* carrying only the vector and *B. burgdorferi* strain Sh-2-82. Each strip was incubated with a different human serum except for one strip from each gel which was incubated with anti-pSPR33 serum. This latter strip served as marker for the positions of P28 and P39. All of 33 sera with IFA titers $\geq$ 1:256 (100%), 13 of 17 sera (76%) with IFA titers = 1:128, and 14 of 44 sera (32%) with titers $\leq$ 1:64 reacted with P39 (see Table 2 below).

Table 2. Summary of human Lyme borreliosis sera tested for reactivity with P39

IFA Titer	No. Sera Tested	No. Sera Reacting with P39	Percent Sera Positive
$\geq$ 1:2048	5	5	100

1:1024 100	8	8
1:512 100	9	9
1:256 100	11	11
1:128 76	17	13
1:64 40	10	4
1:32 55	9	5
≤ 1:16 20	25	5
Total	94	60

Examples of immunoblots for human sera reacting with P39 (arrow 1) are shown in Figure 7. A strongly reacting 58-65 kDa band was observed in the *B. burgdorferi* profile (Fig. 7, band A) for all sera that reacted with P39, but since anti-pSPR33 serum does not react to a band in this region of the gel (Fig. 5), P39 and the 58-65 kDa protein(s) are presumably unrelated. Although P28 appeared to react strongly to some sera (Fig. 7B, band B), for other, less reactive sera, it was not clear if the sera reacted to P28 or to some other protein. This was because these sera also reacted with co-migrating *E. coli* proteins that were detected with a longer autoradiographic exposure (Fig. 7A, band B). Therefore, although it is not clear to what extent P28 actually reacts with human Lyme borreliosis sera, it appears that antibody to P39 was detected in 100% of all sera that had IFA titers ≥ 1:256. Notably, many sera reactive to P39 did not appear to react with the 41 kDa flagellin (Fig. 7A & 7B). In view of this, antibody to P39 could be mistaken as antibody to the flagellin when testing human sera by immunoblot using whole-cell lysates of *B. burgdorferi*. Because P39 was

shown to be specific to *B. burgdorferi* by immunoblot, it is not surprising that control sera, which included sera from 5 ALS patients, 5 syphilitic patients, 5 relapsing fever patients and 10 normal individuals who showed no symptoms of clinical disease, did not react to the cloned P39 protein at a dilution of 1:50 (see Table 3 below). Immunoblot findings for the syphilitic sera are shown in Figure 8. These data suggest that P39 has antigenic specificity for sera collected from patients with Lyme borreliosis. This is despite the fact that both the syphilitic and relapsing fever sera tested had significantly high IFA Lyme borreliosis titers (see Table 3 below), and therefore most likely contained cross-reacting antibodies directed at other *B. burgdorferi* antigens.

Table 3. Summary of IFA titers for control sera that did not react with P39.

Serum description plasma	Lyme IFA	Relapsing fever IFA	Rapid reagin test (1)
Syphilitic			
1	1:128	1:256	1:128
2	1:256	1:1024	1:128
3	1:1024	1:2048	1:128
4	1:512	1:1024	1:64
5	1:128	1:1024	1:32
Relapsing fever			
1	1:1024	1:1024	---
2	1:32	1:512	---
3	1:128	1:512	---
4	1:64	1:512	---
5	1:64	1:1024	---
ALS			
1	1:16	1:64	---
2, 3, 4	<1:16	<1:16	---
5	1:16	1:16	---

Normal			
1, 2, 3, 4	<1:16	1:16	---
5, 6, 7, 8	<1:16	<1:16	---
9, 10	<1:16	1:32	---

1=Portnoy, 1963. Amer. J. Clin. Pathol. 40:473-479

The immunodominance of P39 and this antigens' potential to be a virulence factor of *B. burgdorferi* on account of its immune characteristics and association with infectivity, lead to further characterization of the genetic basis for P39 expression.

Example 4. PSPR 33 Subclone and deletion analysis. Eleven subclones were constructed to determine the approximate position of the P39 locus in the 6.3 kb EcoRI insert in the parent construct pSPR33. The endonucleases EcoRI, ClaI, HindIII, BamHI and PstI were used according to the manufacturer (Boehringer Mannheim Biochemicals) to produce various restriction fragments, which were then ligated to the linearized pBluescript cloning vector (Stratagene) cut with the appropriate enzyme or combination of enzymes. A 4.4 kb EcoRI - ClaI fragment was ligated into the vector and transformed into DH5 alpha Escherichia coli competent cells (Bethesda Research Laboratories) and designated pSPR38 (Fig. 9). A 2.3 kb EcoRI - HindIII fragment produced the subclone pSPR45; a 5.0 kb BamHI fragment produced the subclone pSPR46; a 3.3 kb PstI fragment produced the subclone pSPR44; a 1.4 kb PstI fragment produced the subclone pSPR42. Additional subclones were produced as deletion products by deleting sequences from the HindIII end of the EcoRI - HindIII DNA fragment in the subclone pSPR45. Once digested with HindIII, Dnase was applied for increasing lengths of time to shorten the fragment. The new end was treated with DNA polymerase and nucleotides were added to blunt the end for ligation into linearized, blunt-ended vectors (pBluescript). By successive treatments, the subclones pSPR51, pSPR54, pSPR57, and pSPR59 were

constructed (Fig. 9).

To determine whether the clones were expressing P39, expression assays of the P39 deletion and subclone variants (Fig. 9) were performed with polyclonal anti-P39 serum (anti-pSPR33, previously described), monoclonals A6 and D1 and Western blotted whole-cell lysates. Two monoclonal antibodies to P39 antigen were produced using standard techniques for one of ordinary skill in the art. Escherichia coli cells containing the recombinant pSPR33 were inoculated intraperitoneally into BALB/c laboratory mice. After one month, the mice were boosted with an identical inoculum. One week after the boost, serum samples from the mice were tested by Western blot analysis for anti-P39 antibodies and mice seropositive were again boosted with recombinant E. coli. After three days, spleen were removed. Spleen cells were separated and fused with hybridoma cells SP-20 in HY culture media, 37° C, 8% CO_2. Successful fusions were then cloned by limiting dilution in 96-well microtiter plates. Tissue culture supernatants of positive cell cultures were then tested by Western blot analysis for anti-P39 antibody. Two clones positive for such analysis, designated A6 and D1, were used in subsequent analysis of P39 antigen and the expression of various subclones of pSPR33 as previously described.

To examine various antisera and monoclonal antibodies by Western blot analysis for anti-P39 antibodies, the *E. coli* recombinant with pSPR33 was first lysed by heat in 2-mercaptoethanol and then electrophoresed in a 12.5% SDS-polyacrylamide gel for 6 hr. The gel was then electroblotted with the Towbin system for 3 hr. to transfer the *E. coli* recombinant proteins onto a nitrocellulose membrane. After transfer, the membrane was blocked with TSE-Tween to reduce the nonspecific binding of immunoglobulins. Next the membrane was immersed in the appropriate test serum or monoclonal antibody and incubated at room temperature with rocking for 1 hr. The membrane was then rinsed with water and

incubated next in a solution of ^{125}I-protein A to label antibodies bound to the antigens on the membrane. After incubation and washing off the excess label, the membrane was dried and placed on Kodak XAR-5 film for autoradiographic detection of the anti-P39 antibodies. Similar assays were conducted for the other subclones.

Plasmids pSPR38 and pSPR46 expressed the same amount of P39 as the primary clone pSPR33. This, along with the fact that plasmid pSPR51 expressed P39 whereas pSPR54 did not, we conclude that the gene for P39 was between the RamHI and HindIII sites (Fig. 9, black bar). The amount of P39 associated with cell lysates of clones pSPR51 and pSPR45 is less than the other clones that were P39 positive. This suggested that sequences to the right of the gene locus were important for full expression. P39 was produced by a clone (pSPR46) that contained the insert in the opposite orientation to that of other P39 producing clones (e.g. pSPR38). Therefore, expression or P39 was not dependent on the Lac promoter (Fig. 9, back arrows). The pst1 fragment that was subcloned from pSPR33 and designated pSPR44 (Fig. 9), did not express detectable amounts of P39. Thus, the P39 gene was assumed to be transcribed from left to right. We presume that the additional sequences correspond to the second of two genes that express similar but distinct antigens and that they collectively augment the amount of antibody that binds the 39 kDa band in the immunoblot assay. Because the plasmid pSPR44 did not express any antigens reactive with polyclonal anti-P39 serum, the expression of the second gene located to the right of the black box (Fig. 9) may depend on the transcription of the first gene.

Example 5. <u>DNA sequencing of the gene encoding P39</u>. The DNA sequence (Fig. 10) was determined for the <u>BamHI-HindIII</u> fragment (Fig. 9, black box) by the strategy summarized in Fig. 11b. Essentially, sequence was obtained using primers designed from DNA sequence determined using the universal M13 primer and the subclones pSPR46, pSPR44, and pSPR45, and the Mung Bead

nuclease deletion variants pSPR51, pSPR54, pSPR56, and pSPR57, of plasmid pSPR45. DNA sequence to the right of the HindIII restriction site was determined using primers designed from existing sequence information.

DNA sequence was obtained first by using primers designed for use with the M13 universal primer and available sequence of the cloning vector. The protocol for performing the sequencing reactions was exactly that provided by United States Biochemical (Sequenase - Version 2.0: Step-By-Step Protocols for DNA Sequencing With Sequenase ® Version 2.0 - 5th Edition). Sequencing reactions were run in small plastic centrifuge tubes. Each reaction volume was 10Nl and included primer, buffer and DNA to anneal primer to template. Labeling was done by adding Sequenase, ^{35}S-dATP, and additional buffer. Termination of the A, T, G, and C reactions was done by adding a stop solution. Samples were then heated to 70° - 80°C for two minutes and then 2-3 Nl of each mix was added to each lane of the gel. All sequencing gels were 6% acrylamide - 7M urea - 1 x TBE and were run for 2hr or 4hr. After running, the gels were fixed in 5% acetic acid - 15% methanol to remove urea. Gels were then dried at 80°C under vacuum then placed on Kodak XAR-5 film. Exposed films were then analyzed for autoradiographic bands to determine the sequence. Terminal sequences of each reaction were used to generate new oligonucleotide primers for use in the next sequencing reactions. Therefore, the entire sequences of each strand of DNA were determined through successive extensions using primers determined by previous reactions. By way of example, synthetic primers of 20 nucleotides from a region of SEQ ID no. 1 can be constructed and utilized to sequence about 300 bases. Other primers can then be constructed from the deduced sequence. Such techniques are standard and would be known to one of ordinary skill in the art.

Analysis of the completed DNA sequence (SEQ. ID No. 1) revealed two open reading frames (Fig. 10a). Gene 1 was in frame 1 and gene 2 was in frame 3. No other

significant open reading frames were detected. The DNA sequence has been numbered from the adenine residue of the ATG start codon for the protein encoded by gene 1 because it is assumed that this is the first gene transcribed. This open reading frame (nucleotides 1 to 1020) was confirmed by sequencing the first 15 amino acids of P39 expressed by clone pSPR51. This clone has had gene 2 deleted, and therefore its gene product was not detected during protein sequencing. Gene 1 corresponds to a protein of 339 amino acids with a calculated molecular weight of 36.926 kDa. Because this gene encodes a protein that reacted with all of 10 serum specimens collected from: human Lyme patient but not to 10 normal controls specimens (data not shown), it assumed that this protein is equivalent to P39. Because of the existence of a second gene product with a similar molecular weight that may also bind human serum, it was determined that the P39 antigen as previously described is not one protein but two proteins (39α and 29 β). This is suggested by the expression data shown in Fig. 9, where the P39 signal appears to be enhanced if both genes are present. The open reading frame (nucleotides 1107 to 2132) of gene 2 has been designated p39β. This genes' open reading frame begins 116 nucleotides down stream of p39α and encodes a protein of 341 amino acids (37.506 kDa). A promoter 5' to the start codon in p39α appeared to be present with classic -10 and -35 regions whereas the p39β lacked recognizable promoter sequences. Both genes, however, had putative ribosomal binding sites immediately 5' to the start codons and each was terminated with a TAA codon at positions 1018 and 2130 respectively. The putative promoter and ribosomal binding sites resemble those associated with other genes from *B. burgdorferi* including the opsA-operon and the flagellin gene (Wallich, R., S.E. Moter, M.M. Simon, K. Ebnet, A. Heiberger, and M.D. Kramer, 1990. The *Borrelia burgdorferi* flagellum-associated 41 kilodalton antigen (flagellin); molecular cloning, expression and amplification of the gene. Infect

Immun 58:1711-1719). Unlike the genes encoding the flagellin, OspA and OspB, no stem loop structures were detected at the 3' 'end of either p39α and p39β, suggesting termination may be outside what has been sequenced. Nevertheless, in accordance with the transcription termination regions in may bacteria, including *Borrelia*, this region is AT rich, suggesting that termination is in the vicinity of nucleotide 2170.

Comparing the DNA sequence of p39α and p38β by the Needleman and Munsch global alignment program (Needleman and Munsch, J. Mol. Biol.; 148:443-53 (1970)), indicates that these genes have 62% DNA sequence similarity. No significant sequence similarity was detected between the P39 genes and either the OspA-OspB operon or the flagellin gene. Codon preference and G + C content analysis of the p39 operon indicated that there were no significant differences between it and the other *Borrelia* genes.

Example 6. Determination of p39α and p39β transcript size. Northern blot analysis (Fig. 12) of total RNA from *B. burgdorferi* strains B31 and Sh-2-82 were probed with a PstI-HindIII fragment internal to the p39α and p39β loci (Fig. 11). This probe detected a single 2.35 kb message, and tends to confirm that the P39α and β mRNA is polycistronic and that p39α and p39β constitute an operon (p39). This conclusion is supported by the DNA sequence data described above which shows that p39β does not appear to have a recognizable promoter. Furthermore, this explains why clones that carry an intact p39β but lack the promoter for p39α (eg. pSPR44), do not express antigens reactive with polyclonal anti-P39 serum (anti-pSPR33) (Fig. 9). As a control for specificity, total RNA from E. coli was shown not to hybridize to the PstI-HindIII *Borrelia* fragment (Fig. 12). The amino acid composition of P39α and P39β are similar (SEQ. ID. No. 2 and 3, Table 4), although distinct from the amino acid composition of OspA and OspB. P39 and P39β contained comparatively much larger amounts of isoleucine, proline, arginine, phenylalanine, tyrosine, and methionine.

Furthermore, lysine and threonine, which are present in large amounts in OspA and OspB, constitute a much smaller proportion of P39α and P39β. Between P39α and P39β, the major difference was the 3 cysteine residues in the later protein and 4 histidine residues in the former protein (Table 4).

Table 4. Amino acid composition of proteins encoded by the P39 operon

	P39α(%)	P39β(%)
Alanine	25 (7.4)	22 (6.5)
Cysteine	1 (0.3)	3 (0.9)
Aspartic acid	20 (5.9)	21 (6.2)
Glutamic acid	26 (7.7)	21 (6.2)
Phenylalanine	16 (4.7)	16 (4.7)
Glycine	33 (9.7)	32 (9.4)
Histidine	4 (1.2)	1 (0.3)
Isoleucine	37 (10.9)	43 (7.6)
Lysine	30 (8.8)	26 (7.6)
Leucine	32 (9.4)	26 (7.6)
Methionine	5 (1.5)	6 (1.8)
Asparagine	17 (5.0)	21 (6.2)
Proline	8 (2.4)	7 (2.1)
Glutamine	4 (1.2)	7 (2.1)
Arginine	7 (2.1)	9 (2.6)
Serine	29 (8.6)	30 (8.8)
Threonine	12 (3.5)	5 (1.5)
Valine	18 (5.3)	25 (7.3)
Tryptophan	1 (0.3)	2 (0.6)
Tyrosine	14 (4.1)	18 (5.3)
	339	341

* * * * * *

P39β, line OspA and OspB, has a classic signal peptide including the putative cleavage site defined by the tetrapeptide Leu-X-X-Cys (Fig. 13), where X usually represents any neutral amino acid. For P39β, the leu residue is at position 12 and the cysteine at position 15

(SEQ. ID. No. 1). Although P39α also has a hydrophobic N-terminus (Fig. 14) and a cysteine at a similar position (position 18), this protein does not have the tetrapeptide, suggesting that its putative signal sequence is processed in a different manner to that of the corresponding region in P39β. Because P39α and P39β have a cysteine at close to the same position as the cysteine in OspA and OspB, and it has been predicted that the latter two proteins are acylated at the site, P39α and P39β may also be lipoproteins due to acylation of their N-terminal cysteine residue.

Comparing the amino acid sequence of P39α and P39β revealed 52% sequence identity. This is similar to the reported 53% similarity between OspA and OspB. Surprisingly and in contrast to that found for OspA and OspB, the p39 operon proteins have very similar hydropathy plots (Fig. 14). This, along with the high degree of sequence similarity, indicates that the two proteins share a considerable number of the same epitopes having immunogenic properties. Antiserum raised to OspA will react to OspB, indicating proteins like P39α and P39β with significant identity at the amino acid level will share cross-reactive epitopes.

The genetic element encoding the immunodominate antigen P39 was identified and sequenced. This element was shown to be two genes that constituting an operon encoding two similar sized proteins, P39α and P39β, that have considerably amino acid sequence similarity. This is the first report of an operon encoding putative membrane proteins that has a chromosomal origin in *B. burgdorferi*. It is assumed that both the α and β forms contribute to the signal when antibody from infected animals binds the P39 band in Western blots (Simpson, W.J., W. Burgdorfer, M.E. Schrumpf, R.H. Karstens, and T.G. Schwan, 1991. Antibody to a 39 kDa *Borrelia burgdorferi* antigen (P39) as a marker for infection in experimentally and naturally inoculated animals. J Clin Microbio 29:236-243. Simpson, W.J., M.E. Schrumpf, and T.G. Schwan, 1990. Reactivity of

human Lyme borreliosis sera with a 39-kilodalton antigen specific to *Borrelia burgdorferi*. *J Clin Microbiol 28:1329-1337*). This raises the question of whether all Lyme serum reacts equally well to both α and β forms or whether some serum reacts to one and not the other.

The function of P39α and P39β is not known, but several characteristics, deduced from the predicted amino acid sequence suggests several possibilities. These proteins exhibit alternating hydrophobic and hydrophilic regions, characteristic of an amphophilic or transmembrane protein. In accordance with a membrane location, immune electron microscopy analyses of *B. burgdorferi* with monoclonal antibody A6 indicates that the P39 antigen is in or associated with the membranes (unpublished data).

P39β resembles OspA and OspB in that it has typical signal sequence and cleavage site at the first cysteine residue. Like OspA and OspB, P39β is probably membrane associated and may be acrylated at the N-terminal cysteine. P39α, however, is different with regard to its signal sequence which may not be cleaved because it lacks the type 1 recognition site. If so, P39α may be secreted and therefore the antigen that stimulates the immune response during an infection. This notion would help to explain the earlier observation that anti-P39 antibodies appear to more readily associated with the infected state, because a secreted form could accumulate more rapidly during the early stages of an infection than that associated with cells. (Simpson, W.J., W. Burgdorfer, M.E. Schrumpf, R.H. Karstens, and T.G. Schwan, 1991. Antibody to a 39 kDa *Borrelia burgdorferi* antigen (P39) as a marker for infection in experimentally and naturally inoculated animals. J Clin Microbiol 29:236-243.)

Example 6: <u>Amplification of gene 1 (P39α) and gene 2 (P39β)</u>

To determine the immunoreactiveness both P39α and P39β, gene 1 and gene 2 will be cloned separately and the expression products examined for their reactivity with Lyme immune sera. Standard methodologies for cloning and

expressing each gene can be employed; however, it is preferred to amplify each gene separately using the polymerase chain reactive (PCR) and the primer sequences identified in SEQ. ID Nos. 4-7.

The synthetic oligonucleotide DNA primers described were constructed with an Applied Biosystems Inc. DNA Synthesizer Model 380-B, following the instructions provided by the manufacturer. In this procedure, short chains of nucleotides of a specific order are produced in a concentrated ammonium hydroxide solution. This material is then centrifuged under vacuum to remove the ammonium hydroxide. The dry DNA pellet is then resuspended in TE buffer and the DNA concentration is determined by spectrophotometric absorbance at 260 mm. Concentrations of the DNA primers are then standardized for PCR according to the protocol provided by Perkin-Elmer-Cetus.

To amplify *B. burgdorferi* DNA by PCR using the primers described, the protocol involves mixing the *B. burgdorferi* DNA with either primers 1 and 2 for gene 1 (sequences 4 and 5), primers 1 and 2 for gene 2 (sequences 6 and 7), and sequences 4 and 7 to amplify both genes 1 and 2 together. Also added to the PCR mix is the DNA *Taq* polymerase, buffer, and the mixture of the four nucleotides (dNTPs). This reaction mixture is then subjected to repetitive cycles of three different temperatures to cause denaturing the DNA, annealing of the primers to the template DNA, and extension (polymerization) to produce a new strand of DNA. After 30 cycles using the thermal cycler, the PCR amplification products are examined by running 10 μl of each sample in an electrophoresis agarose gel.

In order that the amplified products can be inserted into known vectors by standard techniques known to a skilled artisan, at the 5' end of each primer, nucleotides will be added that encode for the recognition site for the restriction endonuclease EcoRI (G/AATTC).

The amplified DNA products will be comprised of each gene with the addition of an EcoRI site at each end,

which will allow us to insert this sequence into any one of many available cloning and expression vectors which have only one EcoRI site available, such as pUC, pBluescript, pBR322, etc. The vectors are inserted into host cells to obtain expression of the DNA products. Such techniques are well known to one of ordinary skill in the art.

Next, recombinants having the appropriate sized inserted DNA (1017 bases for gene 1; 1023 bp for gene 2) will be examined by Southern blot analysis to identify the cloned fragments. DNA from recombinants with the presumptive gene 1 or gene 2 will be separated in agarose gels, transferred to nitrocellulose membranes, and probed with the purified EcoRI fragment from pSPR33. Such procedures are standard techniques well known to anyone skilled in the art. After confirming that the amplified cloned fragments are homologous with the pSPR33 insert, the various clones are tested for expression of P39 antigens using standard Western immunoblotting techniques. Rabbit anti-pSPR33 antiserum, anti-P39 monoclonal antibodies (as previously described), and convalescent serum from human Lyme patients will each be reacted with whole-cell lysates of the various clones to identify and obtain expression products of each gene. The synthetic peptides can be mapped to identify specific immunoreactive epitopes, used in bioassays to detect Lyme borreliosis disease or used in vaccines for mammals against Lyme borreliosis disease.

While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be appreciated by one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention and appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(ii) TITLE OF INVENTION: ANTIGENIC PROTEINS OF BORRELIA BURGDORFERI

(iii) NUMBER OF SEQUENCES: 7

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE:

(B) STREET:

(C) CITY:

(D) STATE:

(E) COUNTRY:

(F) ZIP:

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette - 3.5 inches, 1.44 Mb storage

(B) COMPUTER: IBM PC Compatible

(C) OPERATING SYSTEM: MS-DOS

(D) SOFTWARE: WordPerfect 5.0

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME:

(B) REGISTRATION NUMBER:

(C) REFERENCE/DOCKET NUMBER:

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE:

(B) TELEFAX:

(C) TELEX:

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2,307

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: double stranded

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Genomic DNA

(A) DESCRIPTION:

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Borrelia burgdorferi

(B) STRAIN: Sh-2-82

(C) INDIVIDUAL ISOLATE: Sh-2-82, P-6

(D) DEVELOPMENTAL STAGE:

(E) HAPLOTYPE:

(F) TISSUE TYPE:

(G) CELL TYPE:

(H) CELL LINE:

(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:

(A) LIBRARY:

(B) CLONE: pSPR33

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT:

(B) MAP POSITION:

(C) UNITS:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(C) IDENTIFICATION METHOD: By experiment

(D) OTHER INFORMATION: Expression of P-39 antigens

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NUMBER:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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TCCTGATAGT GAATATGCAT TTGATTTATT TAAATCAAAG TTATAAACTA CTAAATATAG  60
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CTTTGTTTGT AAAGGGGAAA TAGTTT ATG AAT AAA ATA TTG TTG TTG ATT TTG  113
                               Met Asn Lys Ile Leu Leu Leu Ile Leu
                                                    1

CTT GAG AGT ATT GTT TTT TTA TCT TGT AGT GGT AAA GGT AGT CTT GGG   161
Leu Glu Ser Ile Val Phe Leu Ser Cys Ser Gly Lys Gly Ser Leu Gly
10                                  20

AGC GAA ATT CCT AAG GTA TCT TTA ATA ATT GAT GGA ACT TTT GAT GAT   209
Ser Glu Ile Pro Lys Val Ser Leu Ile Ile Asp Gly Thr Phe Asp Asp
            30                                          40

AAA TCT TTT AAT GAG AGT GCT TTA AAT GGC GTA AAA AAA GTT AAA GAA   257
Lys Ser Phe Asn Glu Ser Ala Leu Asn Gly Val Lys Lys Val Lys Glu
                                50

GAA TTT AAA ATT GAG CTT GTT TTA AAA GAA TCC TCA TCA AAT TCT TAT   305
Glu Phe Lys Ile Glu Leu Val Leu Lys Glu Ser Ser Ser Asn Ser Tyr
        60                                  70

TTA TCT GAT CTT GAA GGG CTT AAG GAT GCG GGC TCA GAT TTA ATT TGG   353
Leu Ser Asp Leu Glu Gly Leu Lys Asp Ala Gly Ser Asp Leu Ile Trp
                    80

CTT ATT GGG TTT TAT AGA TTT AGC GAT GTG GCC AAG GTT GCG GCT CTT   401
Leu Ile Gly Phe Tyr Agr Phe Ser Asp Val Ala Lys Val Ala Ala Leu
90                                          100

CAA AAT CCC GAT ATG AAA TAT GCA ATT ATT GAT CCT ATT TAT TCT AAC   449
Gln Asn Pro Asp Met Lys Tyr Ala Ile Ile Asp Pro Ile Tyr Ser Asn
                110                                         120

GAT CCT ATT CCT GCA AAT TTG GTG GGC ATG ACC TTT AGA GCT GAA GAG   497
Asp Pro Ile Pro Ala Asn Leu Val Gly Met Thr Phe Arg Ala Gln Glu
                                130

GGT GCA TTT TTA ACG GGT TAT ATT GCT GCA AAA CTT TCT AAA ACA GGT   545
Gly Ala Phe Leu Thr Gly Tyr Ile Ala Ala Lys Leu Ser Lys Thr Gly
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AAA ATT GGA TTT TTA GGG GGA ATA GAA GGC GAG ATA GTA GAT GCT TTT   593
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AGG TAT GGG TAT GAA GCT GGT GCT AAG TAT GCT AAT AAA GAT ATA AAG   641
Arg Tyr Gly Tyr Glu Ala Gly Ala Lys Try Ala Asn Lys Asp Ile Lys
    170                                 180

ATA TCT ACT CAG TAT ATT GGT AGT TTT GCT GAC CTT GAA GCT GGT AGA   689
Ile Ser Thr Gln Tyr Ile Gly Ser Phe Ala Asp Leu Glu Ala Gly Arg
                    190                                     200

AGC GTT GCA ACT AGA ATG TAT TCT GAT GAG ATA GAC ATT ATT CAT CAT   737
Ser Val Ala Thr Arg Met Try Ser Asp Glu Ile Asp Ile Ile His His
                                    210

GCT GCA GGC CTT GGA GGA ATT GGG GCT ATT GAG GTT GCA AAA GAA CTT   785
Ala Ala Gly Leu Gly Gly Ile Gly Ala Ile Glu Val Ala Lys Glu Leu
            220                                 230

GGT TCT GGG CAT TAC ATT ATT GGA GTT GAT GAA GAT CAA GCA TAT CTT   833
Gly Ser Gly His Tyr Ile Ile Gly Val Asp Glu Asp Gln Ala Tyr Leu
                            240

GCT CCT GAC AAT GTA ATA ACA TCT ACA ACT AAA GAT GTT GGT AGA GCT   881
Ala Pro Asp Asn Val Ile Thr Ser Thr Thr Lys Asp Val Gly Arg Ala
    250                                 260

TTA AAT ATT TTT ACA TCT AAC CAT TTA AAA ACT AAT ACT TTC GAA GGT   929
Leu Asn Ile Phe Thr Ser Asn His Leu Lys Thr Asn Thr Phe Glu Gly
                    270                                     280

GGC AAA TTA ATA AAT TAT GGC CTT AAA GAA GGA GTT GTG GGG TTT GTA   977
Gly Lys Leu Ile Asn Tyr Gly Leu Lys Glu Gly Val Val Gly Phe Val
                                    290

AGA AAT CCT AAA ATG ATT TCC TTT GAA CTT GAA AAA GAA ATT GAC AAT  1025
Arg Asn Pro Lys Met Ile Ser Phe Glu Leu Glu Lys Glu Ile Asp Asn
            300                                 310

CTT TCT AGC AAA ATA ATC AAC AAA GAA ATT ATT GTT CCA TCT AAT AAA  1073
Leu Ser Ser Lys Ile Ile Asn Lys Glu Ile Ile Val Pro Ser Asn Lys
                            320

GAA AGT TAT GAG AAG TTT CTT AAA GAA TTT ATT TAA ATAAAGAATC AATTTATATA
1129  Gly Ser Tyr Glu Lys Phe Leu Lys Glu Phe Ile ***
          330

TTTTATTTTT AAGTATAAAA AACACATTGG TTTTGTTTGA ATAATTGAAA TGGAGAAGTG 1189

CTTTAT ATG AGA ATT GTA ATT TTT ATA TTC GGT ATT TTT TTG ACT TCT    1237
       Met Arg Ile Val Ile Phe Ile Phe Gly Ile Leu Leu Thr Ser
       1                                   10
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TGC TTT AGT AGA AAT GGA ATA GAA TCT AGT TCA AAA AAA ATT AAG ATA   1285
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TCC ATG TTG GTA GAT GGT GTT CTT GAC GAC AAA TCT TTT AAT TCT AGT   1333
Ser Met Leu Val Asp Gly Val Leu Asp Asp Lys Ser Phe Asn Ser Ser
                                40

GCT AAT GAG GCT TTA TTA CGC TTG AAA AAA GAT TTT CCA GAA AAT ATT   1381
Ala Asn Glu Ala Leu Leu Arg Leu Lys Lys Asp Phe Pro Glu Asn Ile
            50                                      60

GAA GAA GTT TTT TCT TGT GCT ATT TCT GGA GTT TAT TCT AGT TAT GTT   1429
Glu Glu Val Phe Ser Cys Ala Ile Ser Gly Val Tyr Ser Ser Tyr Val
                        70

TCA GAT CTT GAT AAT TTA AAA AGG AAT GGC TCA GAC TTG ATT TGG CTT   1477
Ser Asp Leu Asp Asn Leu Lys Arg Asn Gly Ser Asp Leu Ile Trp Leu
    80                                  90

GTA GGG TAC ATG CTT ACG GAT GCA TCT TTA TTG GTT TCA TCG GAG AAT   1525
Val Gly Tyr Met Leu Thr Asp Ala Ser Leu Leu Val Ser Ser Glu Asn
                    100                                     110

CCA AAA ATT AGC TAT GGA ATA ATA GAT CCC ATT TAT GGT GAT GAT GTT   1573
Pro Lys Ile Ser Tyr Gly Ile Ile Asp Pro Ile Tyr Gly Asp Asp Val
                                120

CAG ATT CCT GAA AAC TTG ATT GCT GTT GTT TTC AGA GTA GAG CAA GGT   1621
Gln Ile Pro Glu Asn Leu Ile Ala Val Val Phe Arg Val Glu Gln Gly
            130                                     140

GCT TTT TTG GCT GGC TAT ATT GAC GCC AAA AAA AGC TTT TCT GGC AAA   1669
Ala Phe Leu Ala Gly Thr Ile Ala Ala Lys Lys Ser Phe Ser Gly Lys
                        150

ATA GGT TTT ATA GGG GGA ATG AAG GGT AAT ATA GTA GAT GCA TTT CGC   1717
Ile Gly Phe Ile Gly Gly Met Lys Gly Asn Ile Val Asp Ala Phe Arg
                                170

ACT GGT TAT GAA TCT GGA GCA AAG TAT GCT AAT AAA GAT ATA GAG ATT   1765
Thr Gly Tyr Glu Ser Gly Ala Lys Tyr Ala Asn Lys Asp Ile Glu Ile
                    180                                     190

ATA AGT GAA TAT TCC AAT TCT TTT TCC GAT GTT GAT ATT GGT AGA ACC   1813
Ile Ser Glu Tyr Ser Asn Ser Phe Ser Asp Val Asp Ile Gly Arg Thr
                                200

ATA GCT AGT AAA ATG TAT TCT AAA GGG ATA GAT GTA ATT CAT TTT GCA   1861
Ile Ala Ser Lys Met Tyr Ser Lys Gly Ile Asp Val Ile His Phe Ala
            210                                     220

GCT GGT TTA GCA GGA ATT GGT GTT ATT GAG GCA GCA AAA AAC CTT GGC   1909
Ala Gly Leu Ala Gly Ile Gly Val Ile Glu Ala Ala Lys Asn Leu Gly
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GAT GGT TAC TAT GTT ATT GGA GCC GAT CAG GAT CAG TCA TAT CTT GCT   1957
Asp Gly Tyr Tyr Val Ile Gly Ala Asp Gln Asp Gln Ser Tyr Leu Ala
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CCT AAA AAT TTT ATT ACT TCT GTT ATA AAA AAC ATT GGG GAC GCA TTG   2005
Pro Lys Asn Phe Ile Thr Ser Val Ile Lys Asn Ile Gly Asp Ala Leu
                    260                                 270

TAT TTG ATT ACT GGC GAA TAT ATT AAA AAT AAT AAT GTT TGG GAA GGT   2053
Tyr Leu Ile Thr Gly Glu Tyr Ile Lys Asn Asn Asn Val Trp Glu Gly
                                280

GGA AAA GTT GTT CAA ATG GGA TTA AGA GAT GGT GTT ATT GGG CTG CCT   2101
Gly Lys Val Val Gln Met Gly Leu Arg Asp Gly Val Ile Gly Leu Pro
            290                                 300

AAT GCG AAT GAA TTT GAA TAC ATA AAA GTT CTT GAG AGA AAA ATA GTC   2149
Asn Ala Asn Glu Phe Glu Tyr Ile Lys Val Leu Glu Arg Lys Ile Val
                        310

AAT AAA GAG ATC ATT GTT CCT TGC AAT CAG GAG GAA TAT GAA ATT TTT   2197
Asn Lys Glu Ile Ile Val Pro Cys Asn Gln Glu Glu Tyr Glu Asn Phe
    320                                 330

ATA AAA CAA ATA TTA AAG TTA TAA ACTTTTGAAA TAGAAAGATT TTAATTTTCC  2251
Ile Lys Gln Ile Leu Lys Leu ***
                    340

AGTTTTTAAT TTTTTAATTA TGTTATATTT ATTGTGTTAT AATAAATAGA AGTACA     2307
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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1109

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: double stranded

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Genomic DNA

(A) DESCRIPTION:

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Borrelia burgdorferi

(B) STRAIN: Sh-2-82

(C) INDIVIDUAL ISOLATE: Sh-2-82, P-6

(D) DEVELOPMENTAL STAGE:

(E) HAPLOTYPE:

(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE: pSPR33

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD: By experiment
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
TCCTGATAGT GAATATGCAT TTGATTTATT TAAATCAAAG TTATAAACTA CTAAATATAG  60
                        -35                         -10

CTTTGTTTGT AAAGGGGAAA TAGTTT ATG AAT AAA ATA TTG TTG TTG ATT TTG  113
                             Met Asn Lys Ile Leu Leu Leu Ile Leu
                             1

CTT GAG AGT ATT GTT TTT TTA TCT TGT AGT GGT AAA GGT AGT CTT GGG   161
Leu Glu Ser Ile Val Phe Leu Ser Cys Ser Gly Lys Gly Ser Leu Gly
10                                  20
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AGC GAA ATT CCT AAG GTA TCT TTA ATA ATT GAT GGA ACT TTT GAT GAT   209
Ser Glu Ile Pro Lys Val Ser Leu Ile Ile Asp Gly Thr Phe Asp Asp
                30                                          40

AAA TCT TTT AAT GAG AGT GCT TTA AAT GGC GTA AAA AAA GTT AAA GAA   257
Lys Ser Phe Asn Glu Ser Ala Leu Asn Gly Val Lys Lys Val Lys Glu
                                    50

GAA TTT AAA ATT GAG CTT GTT TTA AAA GAA TCC TCA TCA AAT TCT TAT   305
Glu Phe Lys Ile Glu Leu Val Leu Lys Glu Ser Ser Ser Asn Ser Tyr
        60                                          70

TTA TCT GAT CTT GAA GGG CTT AAG GAT GCG GGC TCA GAT TTA ATT TGG   353
Leu Ser Asp Leu Glu Gly Leu Lys Asp Ala Gly Ser Asp Leu Ile Trp
                        80

CTT ATT GGG TTT TAT AGA TTT AGC GAT GTG GCC AAG GTT GCG GCT CTT   401
Leu Ile Gly Phe Tyr Agr Phe Ser Asp Val Ala Lys Val Ala Ala Leu
90                                              100

CAA AAT CCC GAT ATG AAA TAT GCA ATT ATT GAT CCT ATT TAT TCT AAC   449
Gln Asn Pro Asp Met Lys Tyr Ala Ile Ile Asp Pro Ile Tyr Ser Asn
                    110                                     120

GAT CCT ATT CCT GCA AAT TTG GTG GGC ATG ACC TTT AGA GCT GAA GAG   497
Asp Pro Ile Pro Ala Asn Leu Val Gly Met Thr Phe Arg Ala Gln Glu
                                130

GGT GCA TTT TTA ACG GGT TAT ATT GCT GCA AAA CTT TCT AAA ACA GGT   545
Gly Ala Phe Leu Thr Gly Tyr Ile Ala Ala Lys Leu Ser Lys Thr Gly
            140                                 150

AAA ATT GGA TTT TTA GGG GGA ATA GAA GGC GAG ATA GTA GAT GCT TTT   593
Lys Ile Gly Phe Leu Gly Gly Ile Glu Gly Glu Ile Val Asp Ala Phe
                        160

AGG TAT GGG TAT GAA GCT GGT GCT AAG TAT GCT AAT AAA GAT ATA AAG   641
Arg Tyr Gly Tyr Glu Ala Gly Ala Lys Try Ala Asn Lys Asp Ile Lys
    170                                         180

ATA TCT ACT CAG TAT ATT GGT AGT TTT GCT GAC CTT GAA GCT GGT AGA   689
Ile Ser Thr Gln Tyr Ile Gly Ser Phe Ala Asp Leu Glu Ala Gly Arg
                    190                                     200


AGC GTT GCA ACT AGA ATG TAT TCT GAT GAG ATA GAC ATT ATT CAT CAT   737
Ser Val Ala Thr Arg Met Try Ser Asp Glu Ile Asp Ile Ile His His
                                210

GCT GCA GGC CTT GGA GGA ATT GGG GCT ATT GAG GTT GCA AAA GAA CTT   785
Ala Ala Gly Leu Gly Gly Ile Gly Ala Ile Glu Val Ala Lys Glu Leu
            220                                 230

GGT TCT GGG CAT TAC ATT ATT GGA GTT GAT GAA GAT CAA GCA TAT CTT   833
Gly Ser Gly His Tyr Ile Ile Gly Val Asp Glu Asp Gln Ala Tyr Leu
                        240
```

```
GCT CCT GAC AAT GTA ATA ACA TCT ACA ACT AAA GAT GTT GGT AGA GCT   881
Ala Pro Asp Asn Val Ile Thr Ser Thr Thr Lys Asp Val Gly Arg Ala
    250                                     260

TTA AAT ATT TTT ACA TCT AAC CAT TTA AAA ACT AAT ACT TTC GAA GGT   929
Leu Asn Ile Phe Thr Ser Asn His Leu Lys Thr Asn Thr Phe Glu Gly
                    270                                     280

GGC AAA TTA ATA AAT TAT GGC CTT AAA GAA GGA GTT GTG GGG TTT GTA   977
Gly Lys Leu Ile Asn Tyr Gly Leu Lys Glu Gly Val Val Gly Phe Val
                                    290

AGA AAT CCT AAA ATG ATT TCC TTT GAA CTT GAA AAA GAA ATT GAC AAT  1025
Arg Asn Pro Lys Met Ile Ser Phe Glu Leu Glu Lys Glu Ile Asp Asn
            300                                     310

CTT TCT AGC AAA ATA ATC AAC AAA GAA ATT ATT GTT CCA TCT AAT AAA  1073
Leu Ser Ser Lys Ile Ile Asn Lys Glu Ile Ile Val Pro Ser Asn Lys
                            320

GAA AGT TAT GAG AAG TTT CTT AAA GAA TTT ATT TAA                  1109
Gly Ser Tyr Glu Lys Phe Leu Lys Glu Phe Ile ***
```

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1198

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: double stranded

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Genomic DNA

(A) DESCRIPTION:

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Borrelia burgdorferi

(B) STRAIN: Sh-2-82

(C) INDIVIDUAL ISOLATE: Sh-2-82, P-6

(D) DEVELOPMENTAL STAGE:

(E) HAPLOTYPE:

(F) TISSUE TYPE:

(G) CELL TYPE:

(H) CELL LINE:

(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE: pSPR33
(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:
(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```
                                                ATAAAGAATC AATTTATATA 20

TTTTATTTTT AAGTATAAAA AACACATTGG TTTTGTTTGA ATAATTGAAA TGGAGAAGTG 80

CTTTAT ATG AGA ATT GTA ATT TTT ATA TTC GGT ATT TTT TTG ACT TCT     128
       Met Arg Ile Val Ile Phe Ile Phe Gly Ile Leu Leu Thr Ser
       1                                   10
TGC TTT AGT AGA AAT GGA ATA GAA TCT AGT TCA AAA AAA ATT AAG ATA   176
Cys Phe Ser Arg Asn Gly Ile Gly Ser Ser Ser Lys Lys Ile Lys Ile
                    20                                      30

TCC ATG TTG GTA GAT GGT GTT CTT GAC GAC AAA TCT TTT AAT TCT AGT   224
Ser Met Leu Val Asp Gly Val Leu Asp Asp Lys Ser Phe Asn Ser Ser
                                    40

GCT AAT GAG GCT TTA TTA CGC TTG AAA AAA GAT TTT CCA GAA AAT ATT   272
Ala Asn Glu Ala Leu Leu Arg Leu Lys Lys Asp Phe Pro Glu Asn Ile
            50                                          60
```

```
GAA GAA GTT TTT TCT TGT GCT ATT TCT GGA GTT TAT TCT AGT TAT GTT   320
Glu Glu Val Phe Ser Cys Ala Ile Ser Gly Val Tyr Ser Ser Tyr Val
                         70

TCA GAT CTT GAT AAT TTA AAA AGG AAT GGC TCA GAC TTG ATT TGG CTT   368
Ser Asp Leu Asp Asn Leu Lys Arg Asn Gly Ser Asp Leu Ile Trp Leu
     80                                  90

GTA GGG TAC ATG CTT ACG GAT GCA TCT TTA TTG GTT TCA TCG GAG AAT   416
Val Gly Tyr Met Leu Thr Asp Ala Ser Leu Leu Val Ser Ser Glu Asn
                100                                 110

CCA AAA ATT AGC TAT GGA ATA ATA GAT CCC ATT TAT GGT GAT GAT GTT   464
Pro Lys Ile Ser Tyr Gly Ile Ile Asp Pro Ile Tyr Gly Asp Asp Val
                            120

CAG ATT CCT GAA AAC TTG ATT GCT GTT GTT TTC AGA GTA GAG CAA GGT   512
Gln Ile Pro Glu Asn Leu Ile Ala Val Val Phe Arg Val Glu Gln Gly
            130                                 140

GCT TTT TTG GCT GGC TAT ATT GAC GCC AAA AAA AGC TTT TCT GGC AAA   560
Ala Phe Leu Ala Gly Thr Ile Ala Ala Lys Lys Ser Phe Ser Gly Lys
                        150

ATA GGT TTT ATA GGG GGA ATG AAG GGT AAT ATA GTA GAT GCA TTT CGC   608
Ile Gly Phe Ile Gly Gly Met Lys Gly Asn Ile Val Asp Ala Phe Arg
     160                                 170

TAT GGT TAT GAA TCT GGA GCA AAG TAT GCT AAT AAA GAT ATA GAG ATT   656
Thr Gly Tyr Glu Ser Gly Ala Lys Tyr Ala Asn Lys Asp Ile Glu Ile
                180                                 190

ATA AGT GAA TAT TCC AAT TCT TTT TCC GAT GTT GAT ATT GGT AGA ACC   704
Ile Ser Glu Tyr Ser Asn Ser Phe Ser Asp Val Asp Ile Gly Arg Thr
                            200

ATA GCT AGT AAA ATG TAT TCT AAA GGG ATA GAT GTA ATT CAT TTT GCA   752
Ile Ala Ser Lys Met Tyr Ser Lys Gly Ile Asp Val Ile His Phe Ala
            210                                 220

GCT GGT TTA GCA GGA ATT GGT GTT ATT GAG GCA GCA AAA AAC CTT GGC   800
Ala Gly Leu Ala Gly Ile Gly Val Ile Glu Ala Ala Lys Asn Leu Gly
                        230

GAT GGT TAC TAT GTT ATT GGA GCC GAT CAG GAT CAG TCA TAT CTT GCT   848
Asp Gly Tyr Tyr Val Ile Gly Ala Asp Gln Asp Gln Ser Tyr Leu Ala
     240                                 250

CCT AAA AAT TTT ATT ACT TCT GTT ATA AAA AAC ATT GGG GAC GCA TTG   896
Pro Lys Asn Phe Ile Thr Ser Val Ile Lys Asn Ile Gly Asp Ala Leu
                260                                 270

TAT TTG ATT ACT GGC GAA TAT ATT AAA AAT AAT AAT GTT TGG GAA GGT   944
Tyr Leu Ile Thr Gly Glu Tyr Ile Lys Asn Asn Asn Val Trp Glu Gly
                            280
```

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GGA AAA GTT GTT CAA ATG GGA TTA AGA GAT GGT GTT ATT GGG CTG CCT    992
Gly Lys Val Val Gln Met Gly Leu Arg Asp Gly Val Ile Gly Leu Pro
            290                                 300

AAT GCG AAT GAA TTT GAA TAC ATA AAA GTT CTT GAG AGA AAA ATA GTC   1040
Asn Ala Asn Glu Phe Glu Tyr Ile Lys Val Leu Glu Arg Lys Ile Val
                        310

AAT AAA GAG ATC ATT GTT CCT TGC AAT CAG GAG GAA TAT GAA ATT TTT   1088
Asn Lys Glu Ile Ile Val Pro Cys Asn Gln Glu Glu Tyr Glu Asn Phe
    320                                 330

ATA AAA CAA ATA TTA AAG TTA TAA ACTTTTGAAA TAGAAAGATT TTAATTTTCC  1142
Ile Lys Gln Ile Leu Lys Leu ***
                    340

AGTTTTTAAT TTTTTAATTA TGTTATATTT ATTGTGTTAT AATAAATAGA AGTACA     1198
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single-Stranded

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Synthetic DNA

(A) DESCRIPTION: A primer from the flanking sequence of 5' to gene 1 of P-39 in B. burgdorferi.

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM: B. burgdorferi

(B) STRAIN: Sh-2-82

(C) INDIVIDUAL ISOLATE: Sh-2-82, P-6

(D) DEVELOPMENTAL STAGE:

(E) HAPLOTYPE:

(F) TISSUE TYPE:

(G) CELL TYPE:

(H) CELL LINE:

(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:

(A) LIBRARY:

(B) CLONE: pSPR33

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE: A primer from the flanking DNA of 5' to gene 1 of P-39 in B. burgdorferi.
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:
ATG AAT AAA ATA TTG TTG 18

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single-Stranded
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Synthetic DNA
(A) DESCRIPTION: A primer from within the insertion sequence of genes 1 and 2 of P-39 in B. burgdorferi.

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Borrelia burgdorferi
(B) STRAIN: Sh-2-82
(C) INDIVIDUAL ISOLATE: Sh-2-82, P-6
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE: pSPR33

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:

(ix) FEATURE: A primer from within the insertion sequence of genes 1 and 2 of P-39 in B. burgdorferi.
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:
AAT AAA TTC TTT AAG AAA 18

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single-Stranded

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Synthetic DNA

(A) DESCRIPTION: A primer from within the insertion sequence of genes 1 and 2 of P-39 in B. burgdorferi.

(iii) HYPOTHETICAL:

(iv) ANTI-SENSE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Borrelia burgdorferi

(B) STRAIN: Sh-2-82

(C) INDIVIDUAL ISOLATE: Sh-2-82, P-6

(D) DEVELOPMENTAL STAGE:

(E) HAPLOTYPE:

(F) TISSUE TYPE:

(G) CELL TYPE:

(H) CELL LINE:

(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:

(A) LIBRARY:

(B) CLONE: pSPR33

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT:

(B) MAP POSITION:

(C) UNITS:

(ix) FEATURE: A primer from within the insertion sequence of genes 1 and 2 of P-39 in B. burgdorferi.

(A) NAME/KEY:

(B) LOCATION:

(C) IDENTIFICATION METHOD:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

ATG AGA ATT GTA ATT TTT 18

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single-Stranded
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Synthetic DNA

(A) DESCRIPTION: A primer from within the insertion sequence of genes 1 and 2 of P-39 in B. burgdorferi.

(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Borrelia burgdorferi
(B) STRAIN: Sh-2-82
(C) INDIVIDUAL ISOLATE: Sh-2-82, P-6
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE: pSPR33

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT:

(B) MAP POSITION:

(C) UNITS:

(ix) FEATURE: A primer from within the insertion sequence of 5' to gene 2 of P39 in B. Burgdorferi.

(A) NAME/KEY:

(B) LOCATION:

(C) IDENTIFICATION METHOD:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NUMBER:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

TAA ATT TAA TAT TTG TTT 18

WHAT IS CLAIMED IS:

1. A substantially pure form of a *Borrelia burgdorferi* protein which has a molecular weight of about 39 kilodalton as determined by SDS-PAGE and which is reactive with mammalian Lyme borreliosis serum.

2. The protein according to claim 1 wherein said protein is P39α or P39β.

3. The protein according to claim 1 wherein the mammal is a human.

4. A substantially pure form a *Borrelia burgdorferi* protein which has a molecular weight of about 28 kilodaltons as determined by SDS-PAGE and which is reactive with mammalian Lyme borreliosis serum.

5. The protein according to claim 4 wherein the mammal is a human.

6. A *Borrelia burgdorferi* protein substantially free of proteins with which it is normally associated that has a molecular weight of about 39 kilodaltons as determined by SDS-PAGE and which is reactive with mammalian Lyme borreliosis serum.

7. The protein according to claim 6 wherein said protein is P39α or P39β.

8. The protein according to claim 6 wherein the mammal is a human.

9. A *Borrelia burgdorferi* protein substantially free of proteins with which it is normally associated that has a molecular weight of about 28 kilodaltons as determined by SDS-PAGE and which is reactive with mammalian Lyme borreliosis serum.

10. The protein according to claim 9 wherein the mammal is a human.

11. A DNA fragment encoding all, or an unique portion, of a *Borrelia burgdorferi* protein, which protein has a molecular weight of about 39 kilodaltons as determined by SDS-PAGE and is reactive with mammalian Lyme borreliosis serum.

12. The DNA fragment according to claim 11 wherein said protein is P39α or P39β.

13. A DNA fragment encoding all, or a unique portion, of *Borrelia burgdorferi* proteins 39α and P39β and which are reactive with mammalian Lyme borreliosis serum.

14. A DNA fragment encoding all, or an unique portion, of a *Borrelia burgdorferi* protein, which protein has a molecular weight of about 28 kilodaltons as determined by SDS-PAGE and is reactive with mammalian Lyme borreliosis serum.

15. A recombinant DNA molecule comprising:

1) said DNA fragment according to claim 11; and

2) a vector.

16. A recombinant DNA molecule comprising:

1) said DNA fragment according to claim 12; and

2) a vector.

17. A recombinant DNA molecule comprising:

1) said DNA fragment according to claim 13, and

2) a vector.

18. A recombinant DNA molecule comprising:

1) said DNA fragment according to claim 14, and

2) a vector.

19. The recombinant DNA molecule according to claim 15 wherein said vector is pBluescript SK.

20. The recombinant DNA molecule according to claim 18 wherein said vector is pBluescript SK.

21. The recombinant DNA molecule according to claim 15 which is pSPR33.

22. A host cell stably transformed with the recombinant DNA molecule according to claim 15 in a manner allowing expression of said protein encoded in said DNA fragment.

23. A host cell stably transformed with the recombinant DNA molecule according to claim 16 in a manner allowing expression of said protein encoded in said DNA fragment.

24. A host cell stably transformed with the recombinant DNA molecule according to claim 17 in a manner allowing expression of said proteins encoded in said DNA fragment.

25. A host cell stably transformed with the recombinant DNA molecule according to claim 18 in a manner allowing expression of said proteins encoded in said DNA fragment.

26. The host cell according to claim 22 wherein said host cell is *Escherichia coli*.

27. The host cell according to claim 18 wherein said host cell is *Escherichia coli*.

28. A method of producing a recombinant *Borrelia burgdorferi* 39 kD protein comprising culturing host cells according to claim 22, in a manner allowing expression of said 39 kD protein, and isolating said 39 kD protein from said host cells.

29. A method of producing a recombinant *Borrelia burgdorferi* 39 kDa protein comprising culturing host cells according to claim 23, in a manner allowing expression of said 39 kD protein, and isolating said 39 kD protein.

30. A method of producing recombinant *Borrelia burgdorferi* 39α and 39β kD proteins comprising culturing host cells according to claim 24, in a manner allowing expression of said 39α and 39β proteins, and isolating said 39α and 39β proteins.

31. A method of producing a recombinant *Borrelia burgdorferi* 28 kD protein comprising culturing host cells according to claim 25, in a manner allowing expression of said 28 kD protein, and isolating said 28 kD protein from said host cells.

32. A complex comprising said protein according to claim 1 bound to a solid support.

33. A purified form of an antibody specific for said protein according to claim 1, or a unique fragment thereof.

34. A purified form of an antibody specific for said protein according to claim 2, or a unique fragment thereof.

35. A purified form of an antibody specific for said protein according to claim 4, or a unique fragment thereof.

36. The antibody according to claim 33 which is monoclonal.

37. The antibody according to claim 35 which is monoclonal.

38. The antibody according to claim 33 which is polyclonal.

39. The antibody according to claim 35 which is polyclonal.

40. A complex comprising said antibody according to claim 33 bound to a solid support.

41. A complex comprising said antibody according to claim 35 bound to a solid support.

42. A vaccine for mammals against Lyme borreliosis disease comprising all, or a unique portion, of a 39 kD *Borrelia burgdorferi* protein, in an amount sufficient to induce immunization against said disease, and a pharmaceutically acceptable carrier.

43. The vaccine according to claim 42 wherein said protein is P39α or P39β.

44. A vaccine for mammals against Lyme borreliosis disease comprising all, or a unique portion, of 39 kD α and β proteins, in an amount sufficient to induce immunization against said disease, and a pharmaceutically acceptable carrier.

45. A vaccine for mammals against Lyme borreliosis disease comprising all, or a unique portion, of a 28 kD *Borrelia burgdorferi* protein, in an amount sufficient to induce immunization against said disease, and a pharmaceutically acceptable carrier.

46. The vaccine according to claim 42 which further comprises an adjuvant.

47. The vaccine according to claim 43 which further comprises an adjuvant.

48. The vaccine according to claim 45 which further comprises an adjuvant.

49. A bioassay for the diagnosis of Lyme borreliosis disease comprising the steps of:

i) coating a surface with all, or a unique portion, of the protein according to claim 1;

ii) contacting said coated surface with serum from a mammal suspected of having Lyme disease; and

iii) detecting the presence or absence of a complex

formed between said protein and antibodies specific therefor present in said serum.

50. The bioassay according to claim 29 wherein said protein is P39α or P39β.

51. A bioassay for the diagnosis of Lyme borreliosis disease comprising the steps of:

i) coating a surface with all, or a unique portion, of the protein according to claim 4;

ii) contacting said coated surface with serum from a mammal suspected of having Lyme disease; and

iii) detecting the presence or absence of a complex formed between said protein and antibodies specific therefor present in said serum.

52. The method according to claim 49 wherein said surface is a gel, a slide, membrane or a microtitration plate.

53. A bioassay for the diagnosis of Lyme borreliosis disease comprising the steps of:

i) coating a surface with antibodies according to claim 33;

ii) contacting said coated surface with serum from a mammal suspected of having Lyme disease; and

iii) detecting the presence or absence of a complex formed between said antibodies and proteins present the said serum.

54. A bioassay for the diagnosis of Lyme borreliosis disease comprising the steps of:

i) coating a surface with antibodies according to claim 34;

ii) contacting said coated surface with serum from a mammal suspected of having Lyme disease; and

iii) detecting the presence or absence of a complex formed between said antibodies and proteins present the said serum.

55. A bioassay for the diagnosis of Lyme borreliosis disease comprising the steps of:

i) coating a surface with antibodies according to claim 35;

ii) contacting said coated surface with serum from a mammal suspected of having Lyme disease; and

iii) detecting the presence or absence of a complex formed between said antibodies and proteins present the said serum.

56. A diagnostic kit comprising a natural or recombinantly produced *Borrelia burgdorferi* 39 kDa proteins and ancillary reagents suitable for use in detecting the presence of antibodies to said proteins in a mammalian tissue sample.

57. The diagnostic kit according to claim 56 wherein said protein is P39α or P39β.

58. The diagnostic kit according to claim 56 wherein said tissue sample to be tested is a blood sample.

59. A method of screening drugs for anti-Lyme borreliosis disease activity comprising contacting said drug with cells contacted with *Borrelia burgdorferi* under conditions such that inhibition of said anti-Lyme activity can be effected.

60. A DNA fragment comprising the nucleotide sequence shown in SEQ ID No. 1 or a mutant thereof.

61. A DNA fragment comprising the nucleotide type sequence shown in SEQ ID No. 2 or a mutant thereof.

62. A DNA fragment comprising the nucleotide sequence shown in SEQ ID No. 3 or a mutant thereof.

63. A recombinant molecule comprising:

1) said DNA fragment according to claim 60; and

2) a vector.

64. A recombinant molecule comprising:

1) said DNA fragment according to claim 61; and

2) a vector.

65. A recombinant molecule comprising:

1) said DNA fragment according to claim 62; and

2) a vector.

66. A host stably transformed with the recombinant DNA molecule according to claim 63 in a matter allowing expression of said proteins encoded in said DNA fragment.

67. A host stably transformed with the recombinant DNA

molecule according to claim 64 in a matter allowing expression of said protein coated in said DNA fragment.

68. A host stably transformed with the recombinant DNA molecule according to claim 65 in a matter allowing expression of said protein coated in said DNA fragment.

69. A method of producing a recombinant *Borrelia burgdorferi* 39 kilodalton proteins comprising culturing host cells according to claim 66, in a manner allowing expression of said proteins, and isolating said proteins from said host cells.

70. A method of producing a recombinant *Borrelia burgdorferi* 39 kilodaltons α protein comprising culturing host cells according to claim 67, in a manner allowing expression of said 39 kilodalton α protein, and isolating said 39 kilodalton α protein from said host cells.

71. A method of producing a recombinant *Borrelia burgdorferi* 39 kilodalton β protein comprising culturing host cells according to claim 68, in a manner allowing expression of said 39 kilodalton β protein, and isolating said 39 kilodalton β protein from said host cells.

72. The proteins produced by the method of claim 69.

73. The protein produced by the method of claim 70.

74. The protein produced by the method of claim 71.

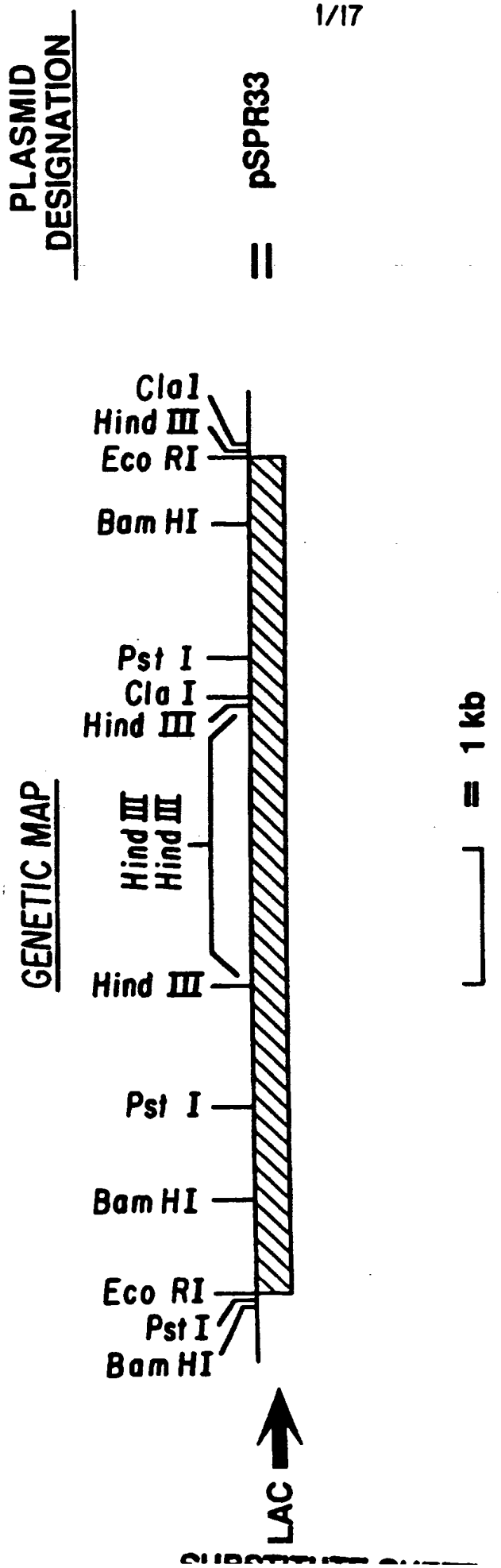


FIG. 1

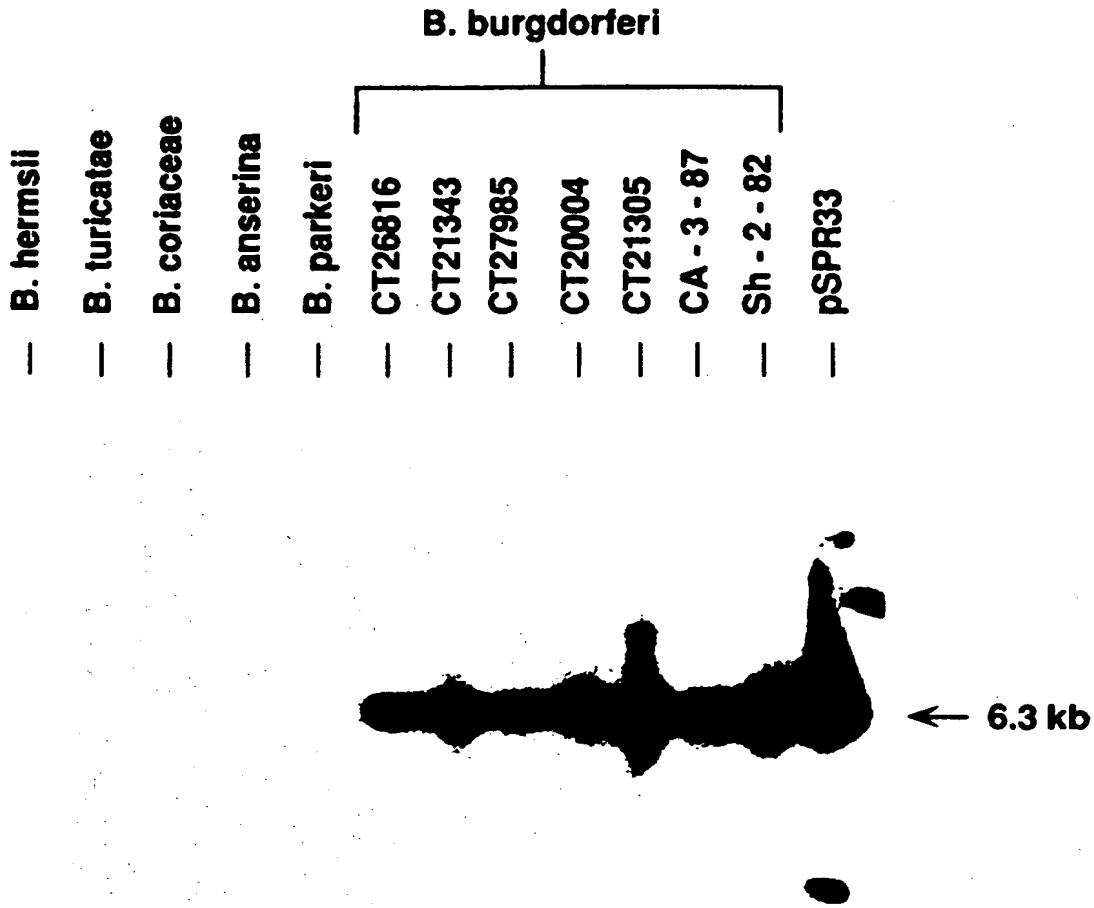


FIG. 2

FIG. 3A

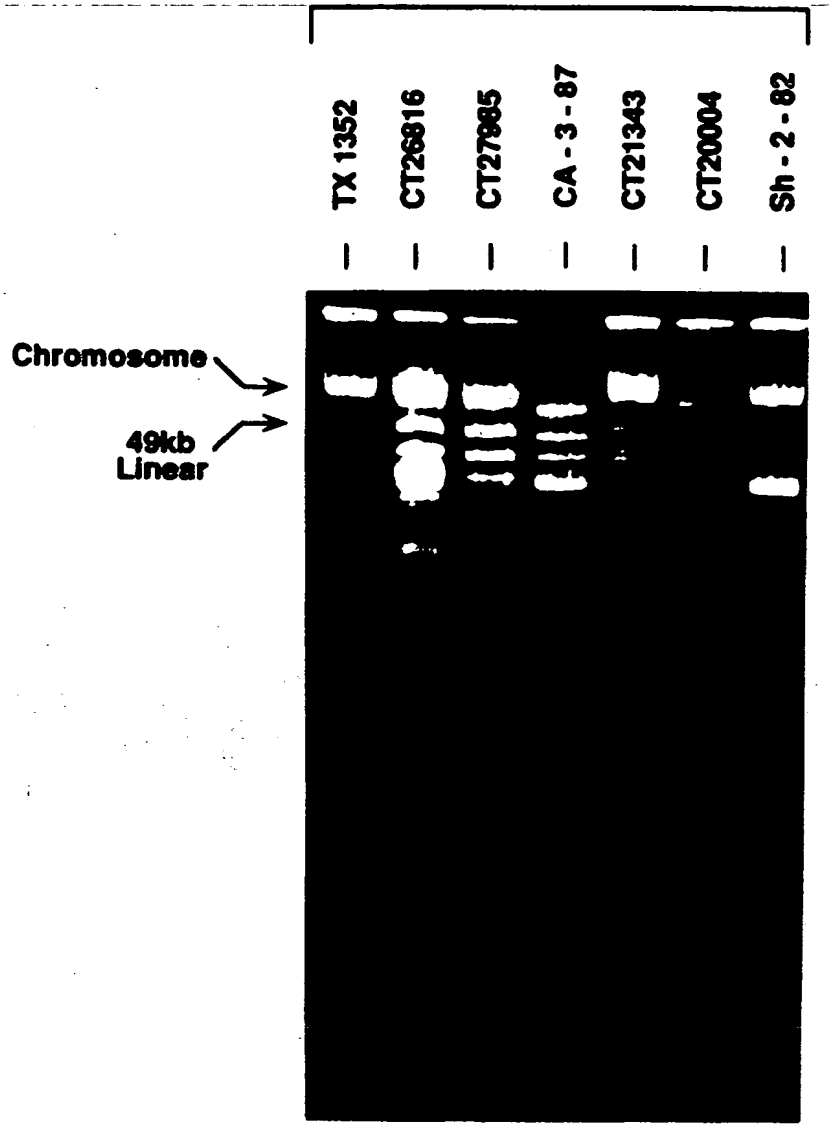
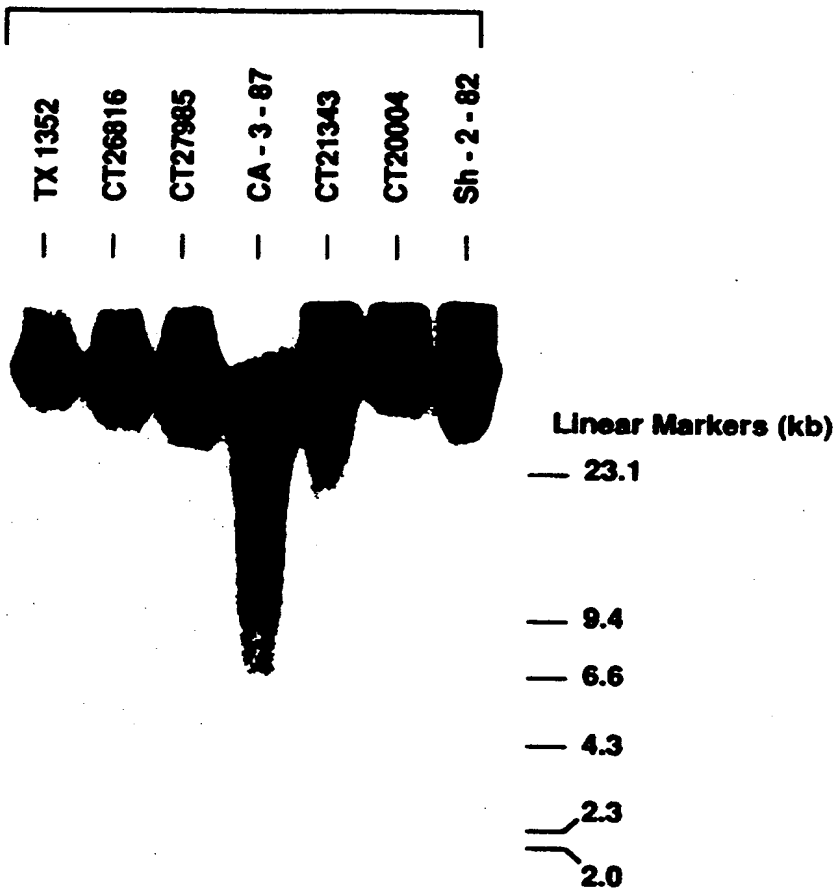


FIG. 3B



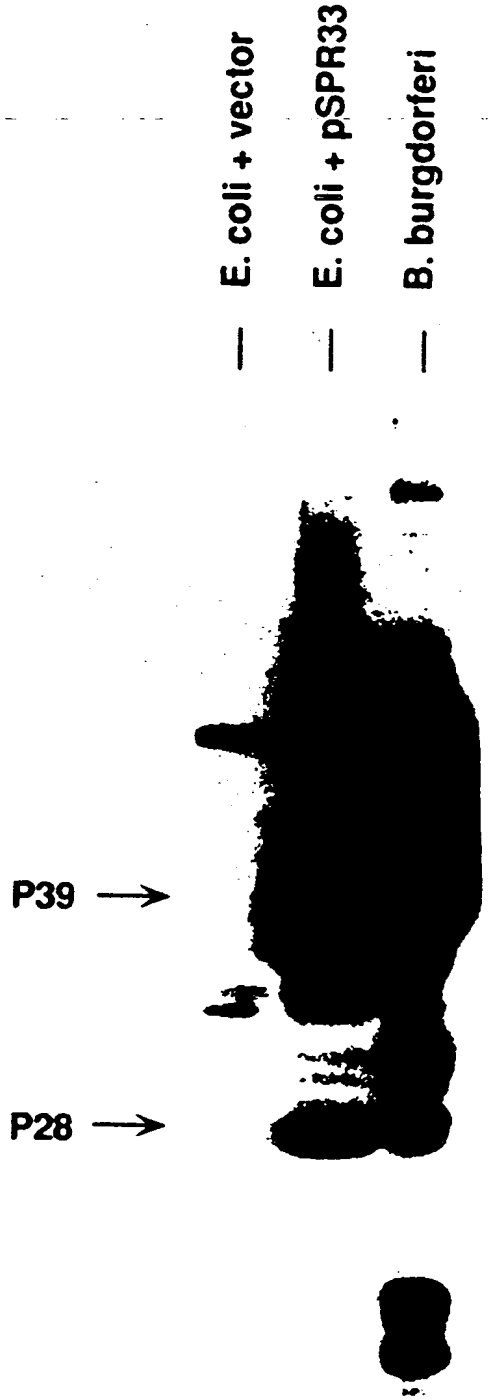


FIG. 4

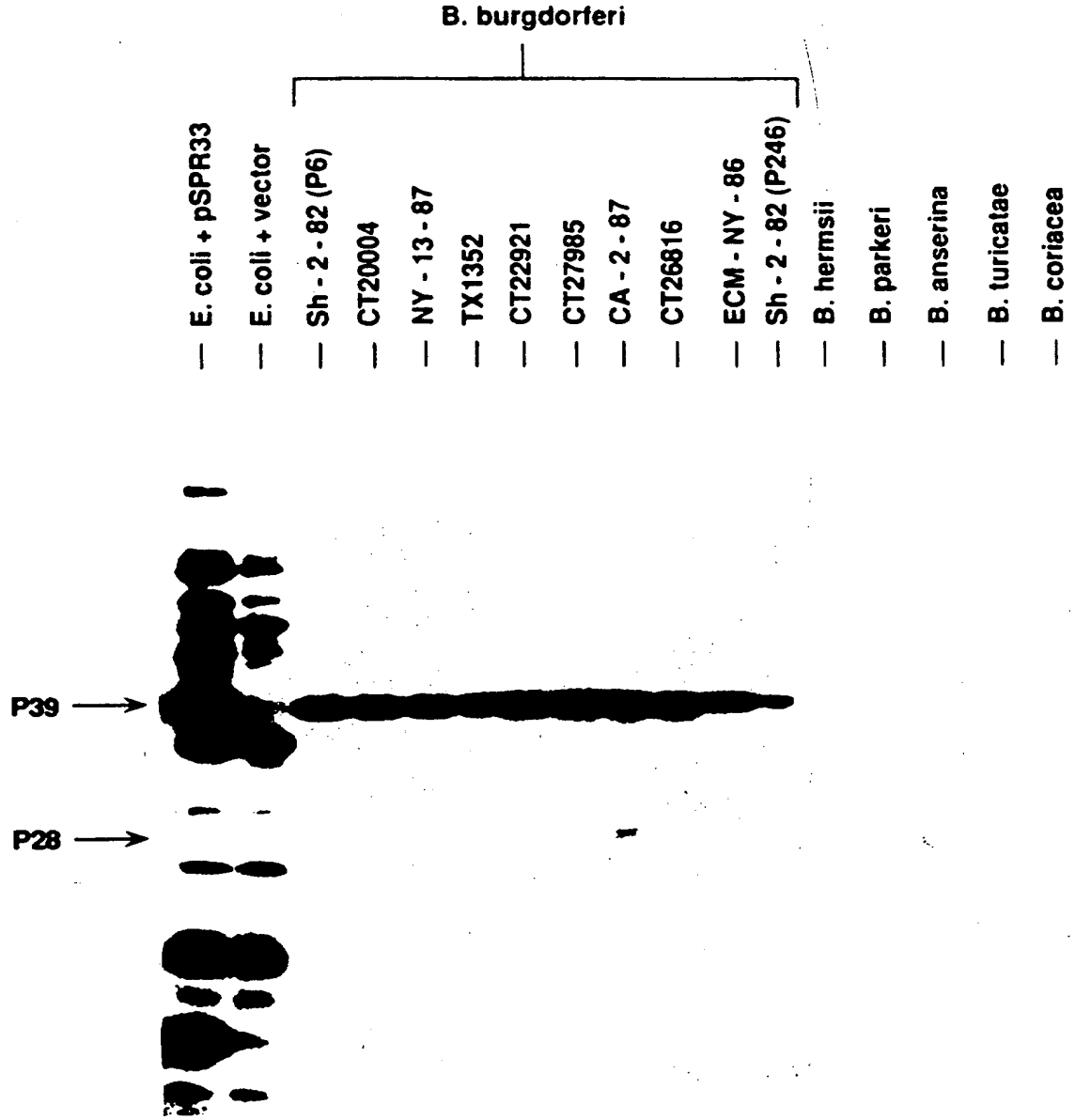


FIG. 5

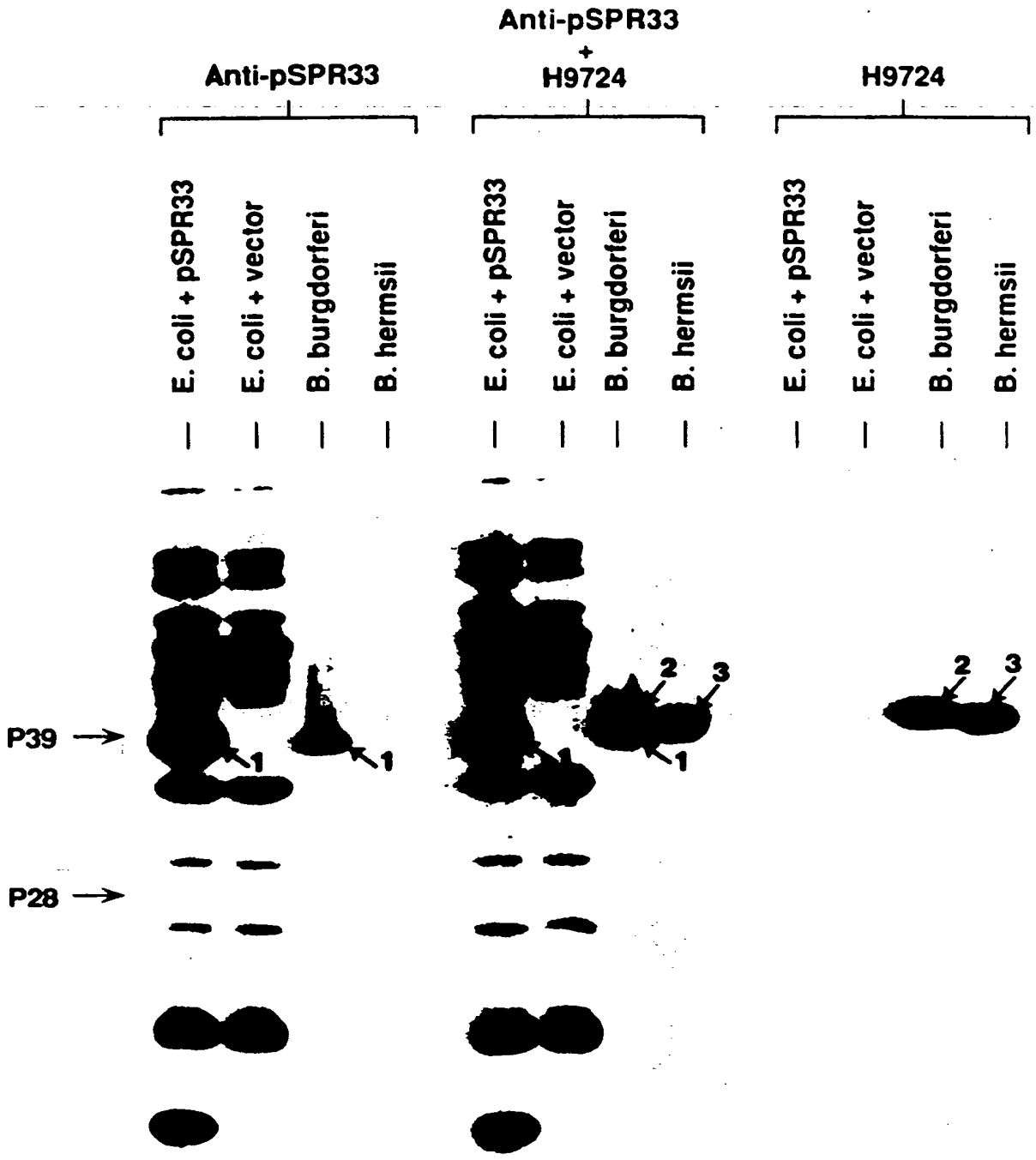


FIG. 6

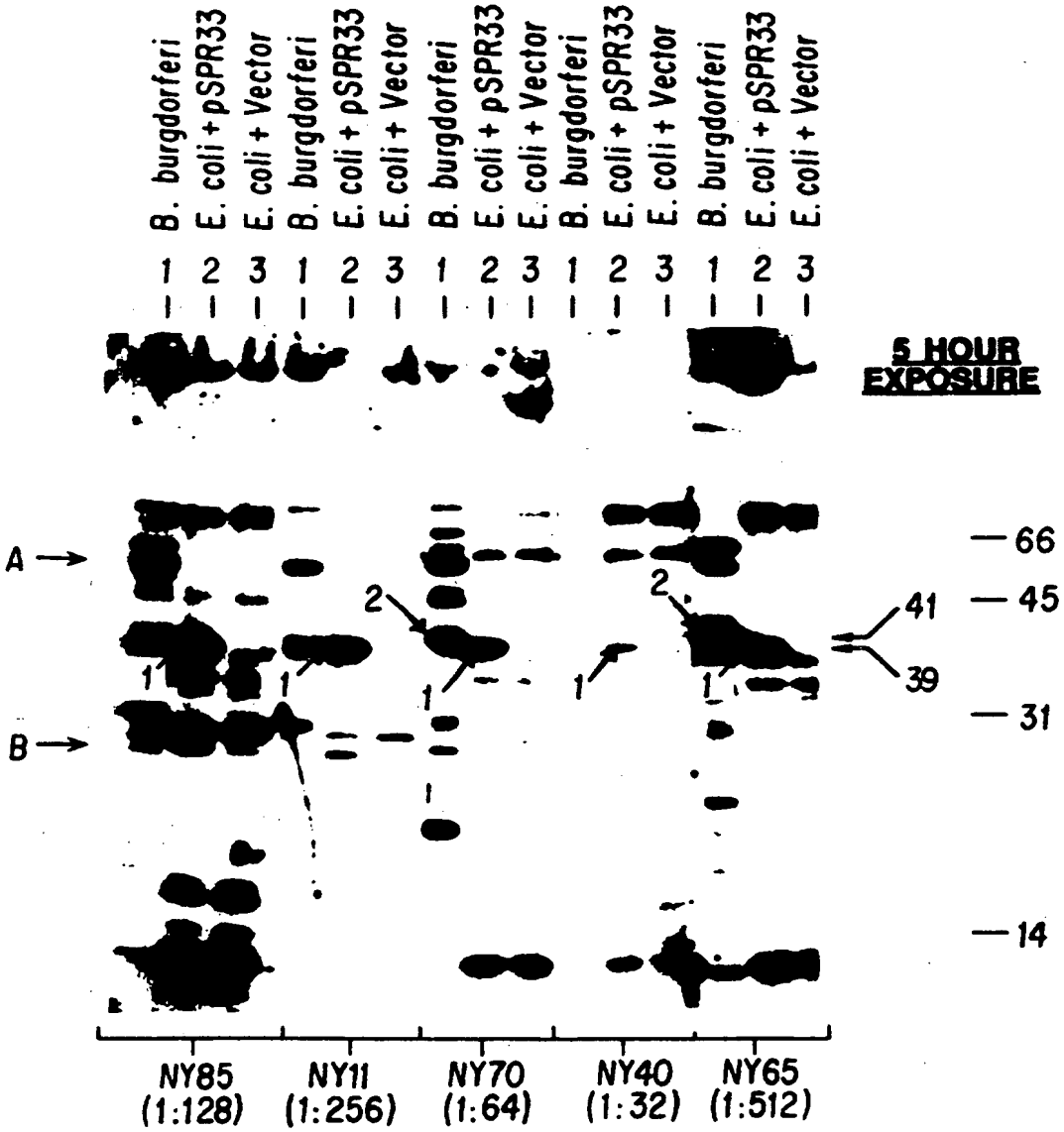


FIG. 7A

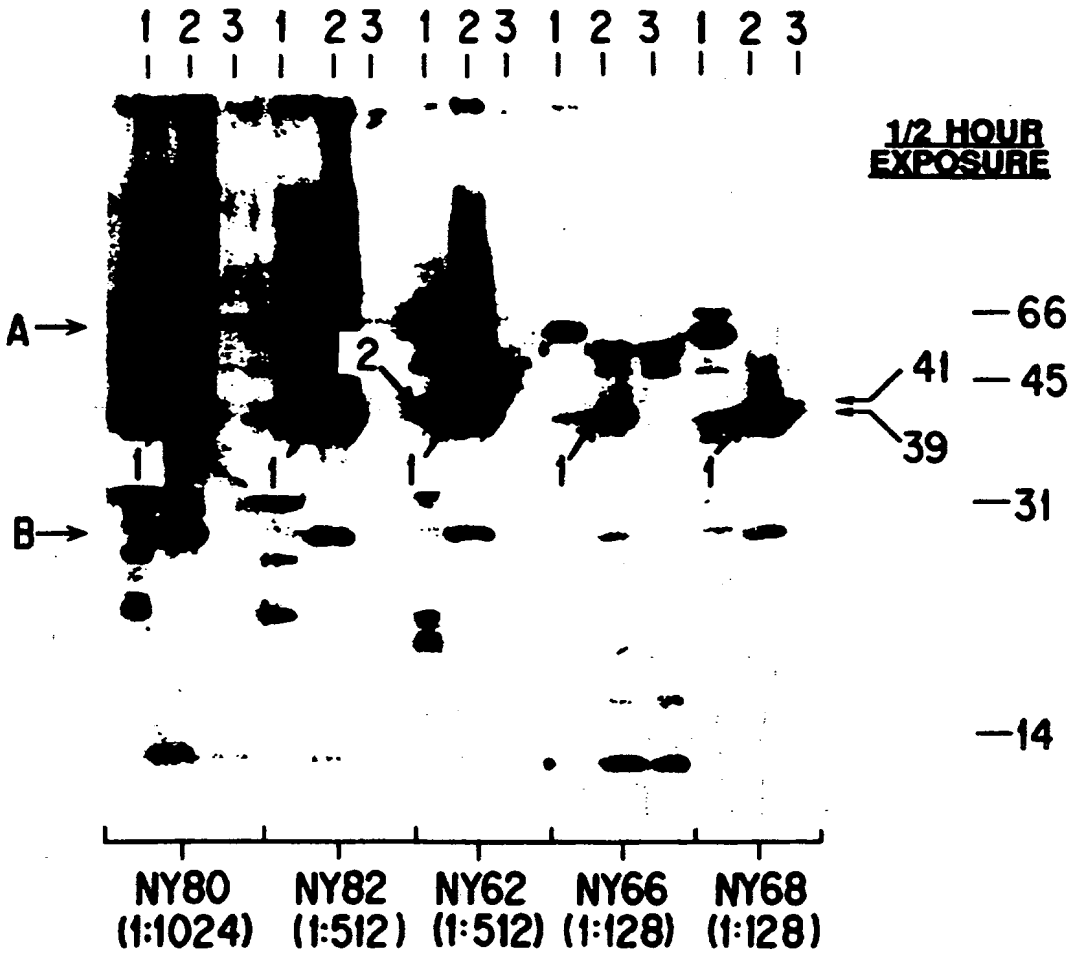


FIG. 7B

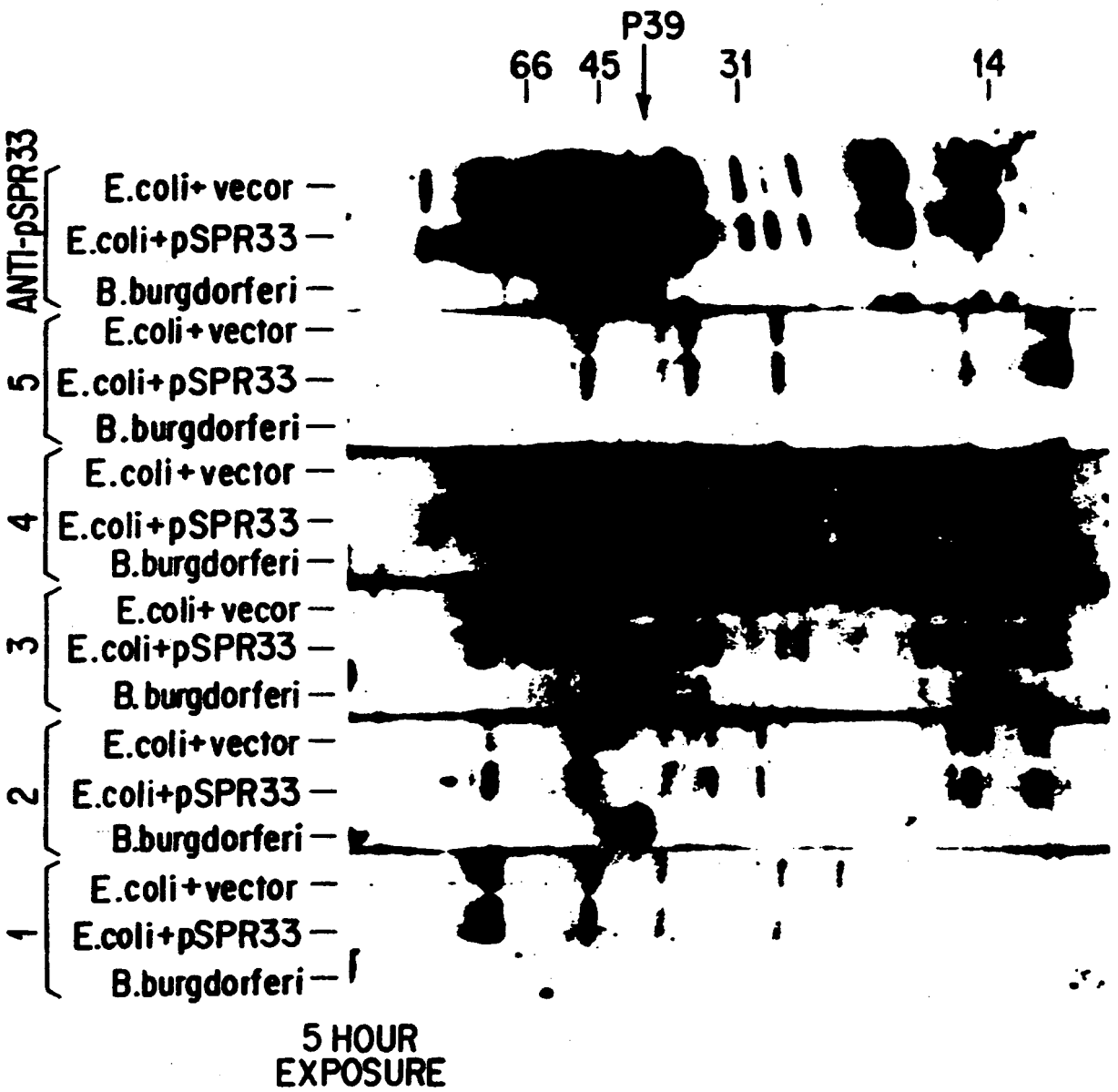


FIG.8

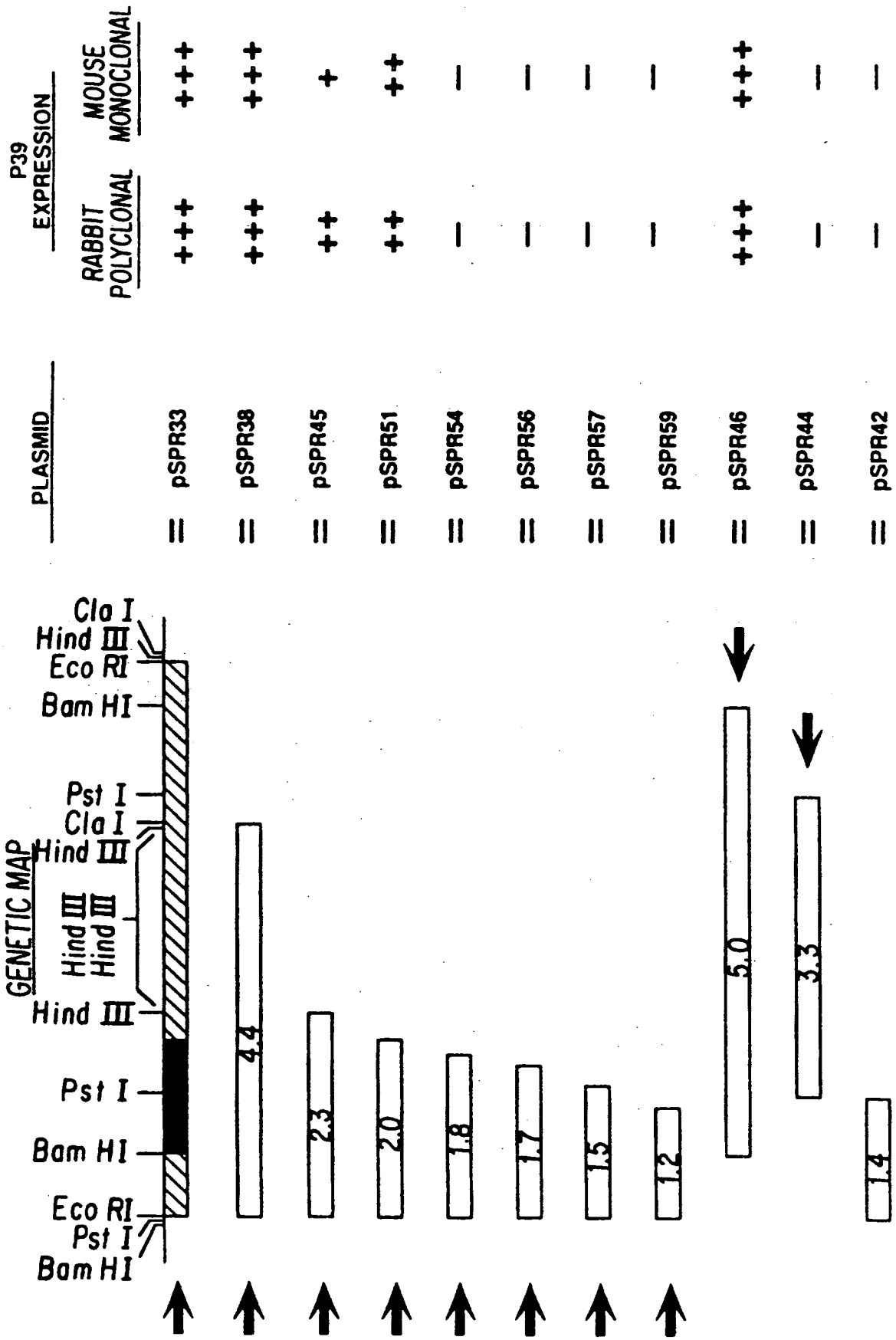


FIG. 9

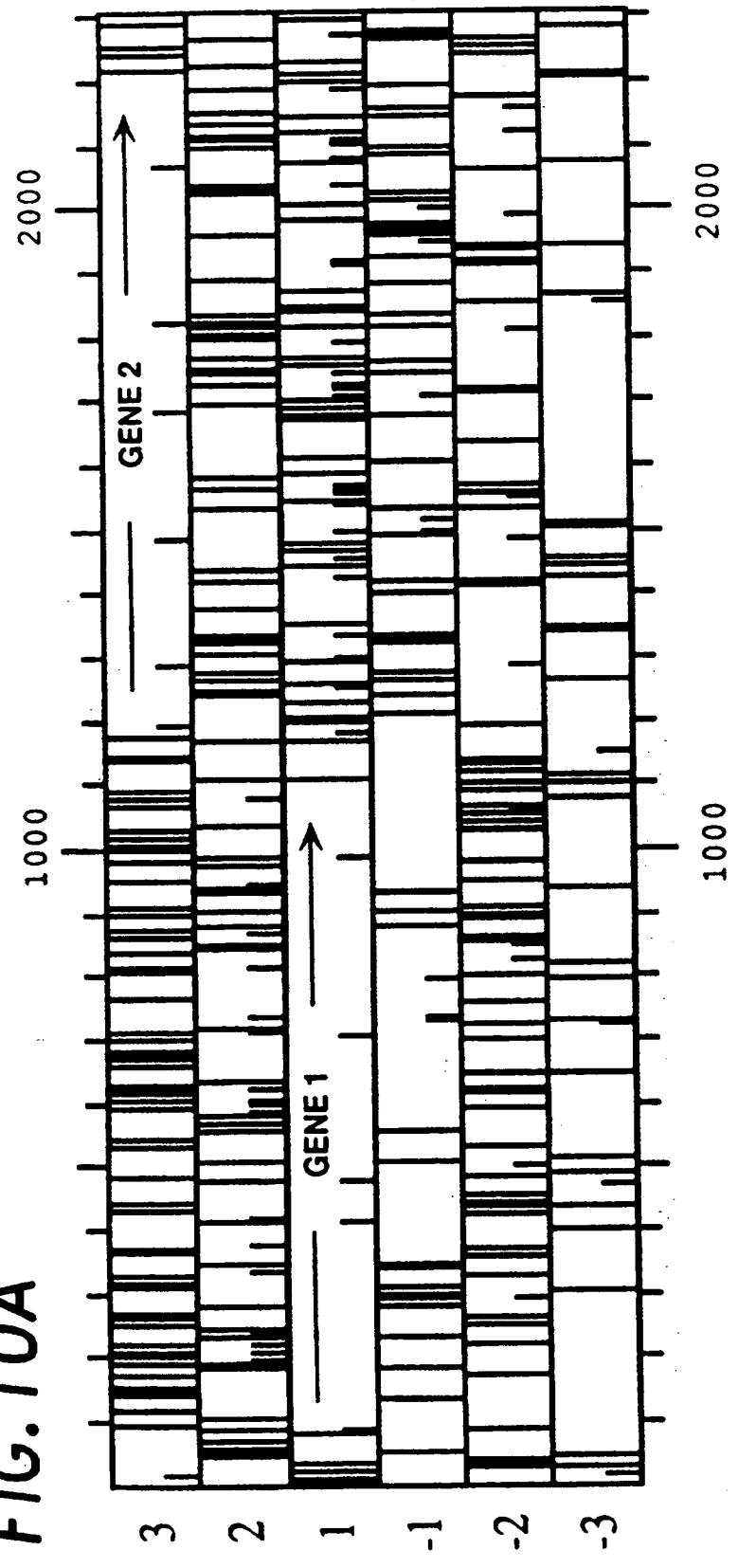


FIG. 10A

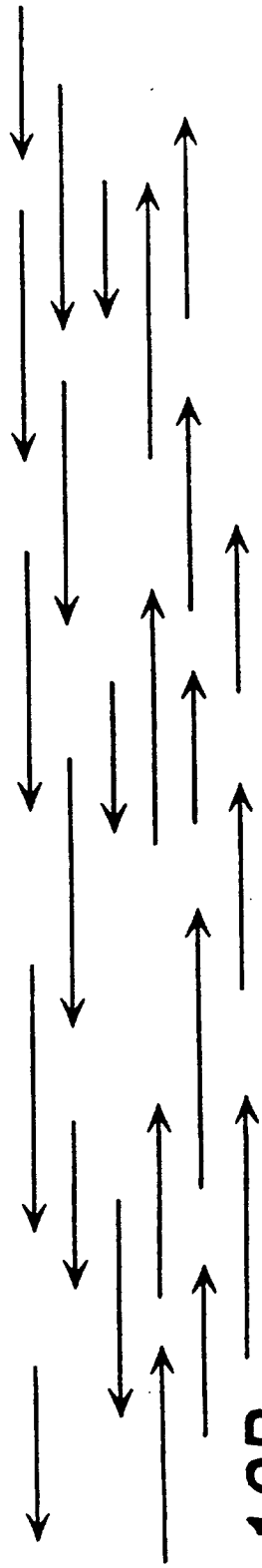


FIG. 10B

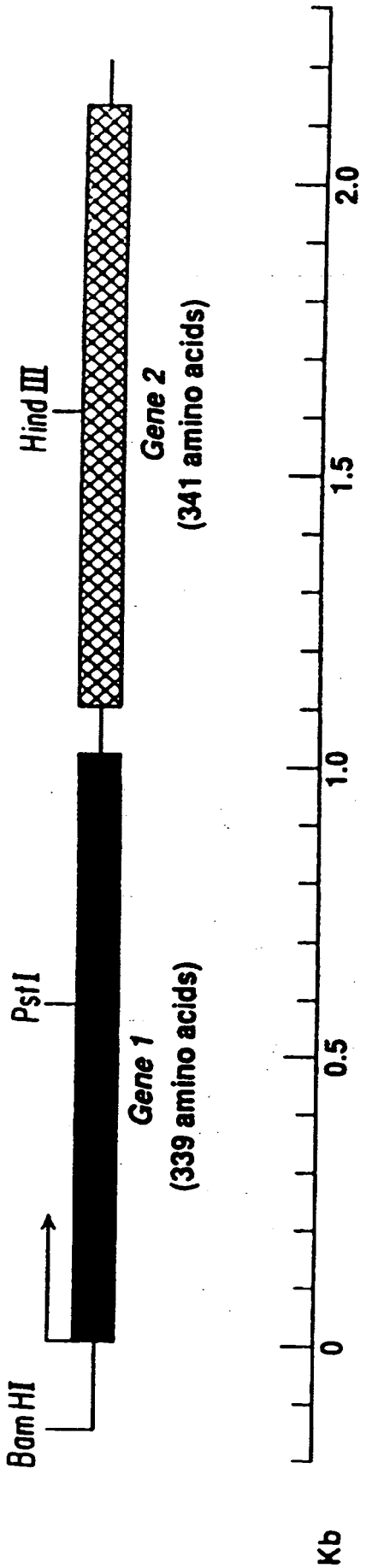


FIG. 11

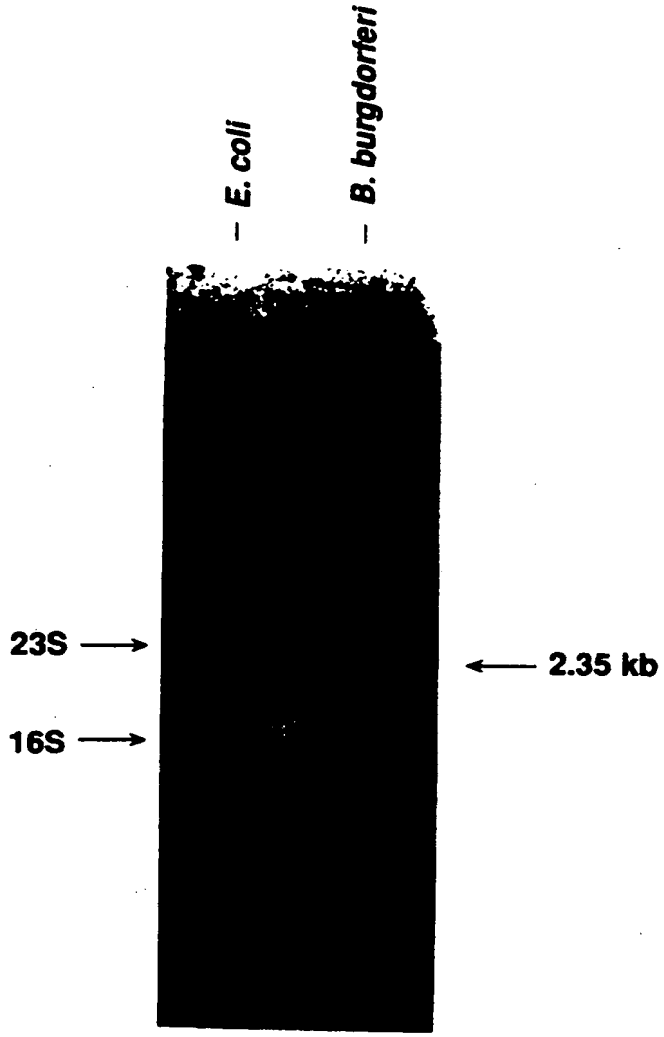


FIG. 12

FIG. 13

	1	5	10	15	
GENE 1	Met	Asn Lys Ile Leu Leu Ile Leu Leu Glu Ser Ile Val Phe Leu Ser	**Cys**	Ser	
GENE 2	Met	Arg Ile Val Ile Phe Ile Phe Gly Ile Leu	**Leu**	Thr Ser	**Cys** Phe Ser Arg Asn
OspA	Met	Lys Lys Tyr Leu Leu Gly Leu Gly Ile Leu Ala	**Leu**	Ile Ala	**Cys** Lys Gln
OspB	Met	Arg Leu Leu Ile Gly Phe Ala Leu Ala Leu Ala	**Leu**	Ile Gly	**Cys** Ala Gln Lys

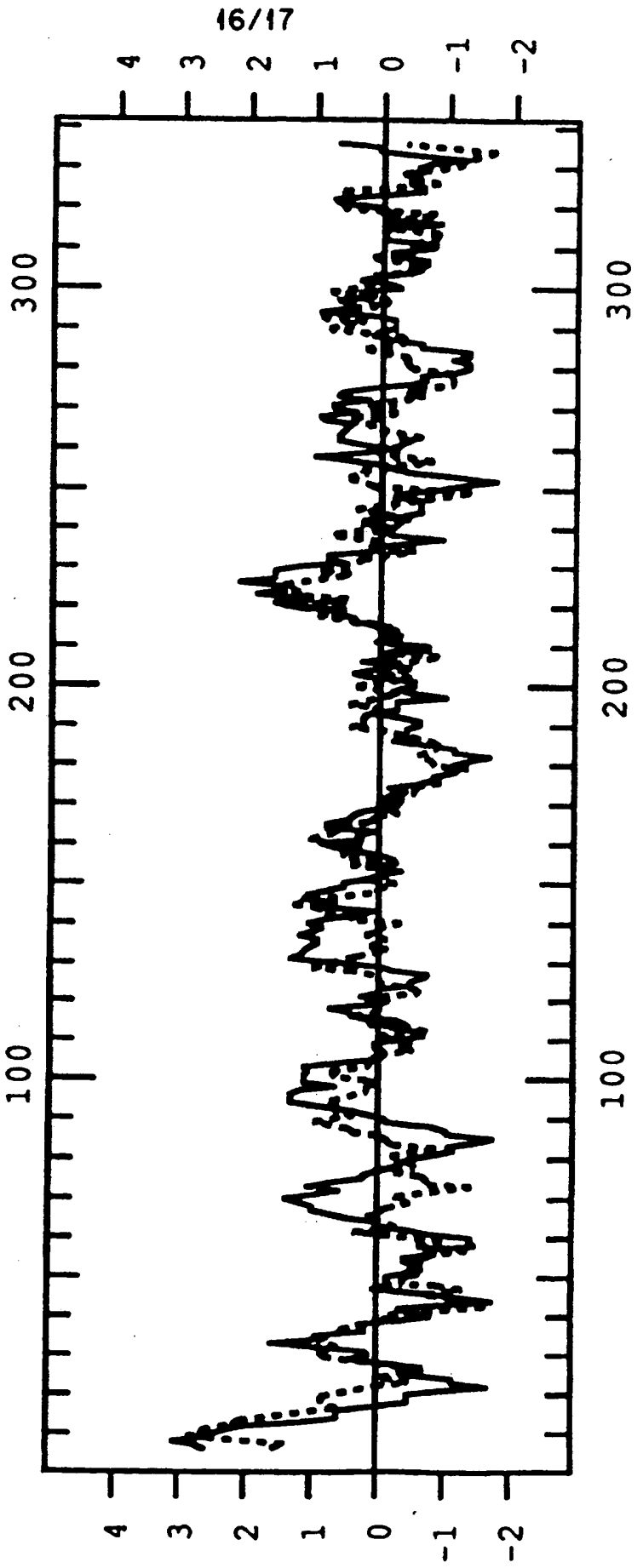


FIG. 14A

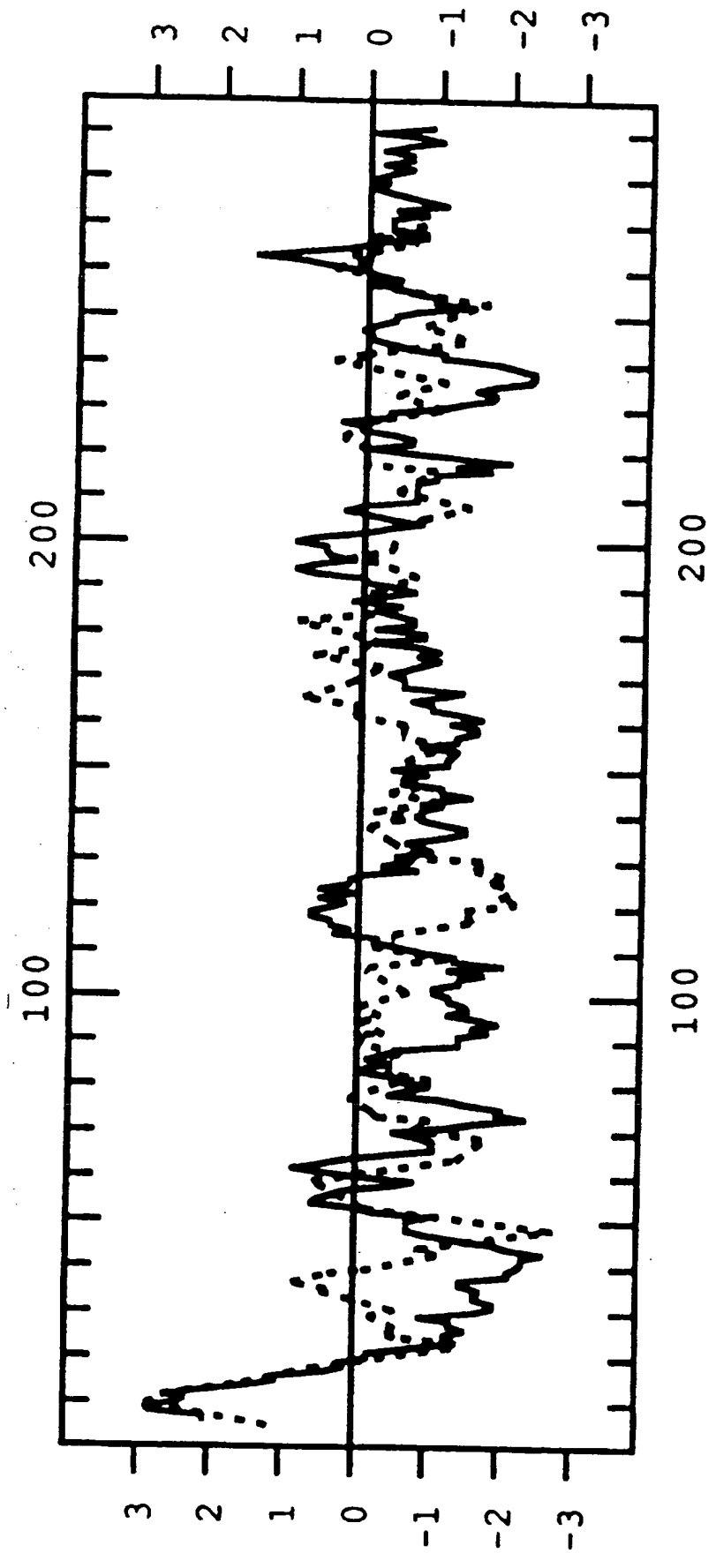


FIG. 14B

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 91/01500

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): A61K 39/00; C12Q 1/00; A01N 37/18; C07K 3/00

U.S.CL 424/85.8; 435/7.22; 514/2; 530/350

II. FIELDS SEARCHED

Minimum Documentation Searched 7

Classification System	Classification Symbols
US: Cl.*	435/7.22 69.1, 91, 172.1, 235, 240.2, 252.3, 320.1; 530/350; 424/85.8, 88, 93; 514/2

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched 8

Chemical Abstract Data Base (cas) 1967-1991

Key words: Borrelia, burgdorferi, antigen, vaccine, assay.

III. DOCUMENTS CONSIDERED TO BE RELEVANT 9

Category *	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No. 13
	See Attached	

* Special categories of cited documents: 10

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

28 May 1991

Date of Mailing of this International Search Report

21 JUN 1991

International Searching Authority

ISA/US

Signature of Authorized Officer

Joan Ellis

Y,P Journal of Clinical Microbiology, Volume 29, No. 2, issued February 1991, W. J. Simpson, et al "Antibody to a 39-Kilodalton Borrelia burgdorferi Antigen (P39) as a Marker for Infection in Experimentally and Naturally Inoculated Animals", pages 236-243, see especially, the abstract. 1-74

A Infection and Immunity, Volume 54, No. 1, issued October 1986, T. R. Hove, et al "Organization of Genes Encoding Two Outer Membrane Proteins of the Lyme Disease Agent Borrelia burdorferi Within a Single Transcriptional Unit", pages 207-212. 1-74

A Journal of Clinical Microbiology, Volume 27, No. 8, issued August 1989, K. E. Hechemy, et al., "Fluoroimmunoassay Studies with Solubilized Antigens from Borrelia burgdorferi", pages 1854-1858. 49-59

A Reviews of Infectious Diseases, Volume 11, Supplement 6, issued September-October 1989, A. G. Barbour, "The Molecular Biology of Borrelia", pages S1470-S1474. 1-74